WO 2004/007712

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DT89 Rec'd PCT/PTO 1 8 JAN 2015

Title: Modulating developmental pathways in plants.

The invention relates to a method to modulate plant growth or development by modifying genes in plants. The invention among others relates to modifying RKS genes or gene products as found in Arabidopsis thaliana or other plants. The 10 different domains of RKS gene products essentially have the following functions: The first domain of the predicted protein structure at the N-terminal end consists of a signal sequence. involved in targeting the protein towards the plasma membrane. Protein cleavage removes this sequence from the final mature 15 protein product (Jain et al. 1994, J. Biol. Chemistry 269: 16306-16310). The second domain consists of different numbers of leucine zipper motifs, and is likely to be involved in protein protein dimerization. The next domain contains a conserved pair of cystein residues, involved in disulphate bridge formation. The next domain consists of 5 (or in the case of RKS3 only 4) leucine rich repeats (LRRs) shown in a gray colour, likely to be involved in ligand binding (Kobe and Deisenhofer 1994, TIBS 19: 415-420). This domain is again bordered by a domain containing a conserved pair of cystein residues involved in disulphate bridge formation often followed by a serine / proline rich region. The next domain displays all the characteristics of a simgle transmembrane domain. At the predicted cytoplasmic site of protein a domain is situated with unknown function, followed by a domain with serine /threonine kinase activity (Schmidt et al. 1997, Development 124: 2049-2062, WO 01/29240). The kinase domain is followed by a domain with unknown function whereas at the Cterminal end of the protein part of a leucine rich repeat is positioned, probably involved in protein-protein interactions.

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Plant homologs of the Arabidopsis RKS genes can be found by comparison of various plant database (see also Table 2) and comprise amongst others:

5 Y14600|SBRLK1|Sorghum bicolor

BF004020|BF004020|EST432518 KV1 Medicago truncatata

AW934655|AW934655|EST353547 tomato

AW617954|AW617954|EST314028 L. pennellii

AA738544|AA738544|SbRLK2 Sorghum bicolor 10 AA738545|AA738545|SbRLK3 Sorghum bicolor

BG595415|BG595415|EST494093 cSTS Solanum tuberosa

AI896277|AI896277|EST265720 tomato

BF643238|BF643238|NF002H05EC1F1045

AA738546|AA738546|SbRLK4 Sorghum bicolor

15 <u>BE658174</u>|BE658174|GM700005A20D5 Gm-r1070 Glycine max

BF520845|BF520845|EST458318 DSIL Medicago truncata

AC069324|AC069324|Oryza sativa

AW761055|AW761055|s170d06.yl Gm-c1027 Glycine max

 $\underline{\texttt{BE352622}} \\ | \\ \texttt{BE352622} \\ | \\ \texttt{WHE0425} \\ \underline{\texttt{G11}} \\ \underline{\texttt{M21ZS}} \\ \\ \texttt{Wheat}$

20 <u>BG647340</u>|BG647340|EST508959 HOGA Medicago truncata AY028699|AY028699|Brassica napus

AW666082|AW666082|sk31h04.v1 Gm-c1028 Glycine max

AA738547|AA738547|SbRLK5 Sorghum bicolor

BG127658|BG127658|EST473220 tomato

25 L27821|RICPRKI|Orvza sativa

BG238468|BG238468|sab51a09.y1 Gm-c1043 Glycine max BG441204|BG441204|GA Ea0012C15f Gossypium arbo.

AW667985|AW667985|GA Ea0012C15 Gossvoium arbore.

AW233982|AW233982|sf32g05.yl Gm-c1028 Glycine max

30 AP003235|AP003235|Oryza sativa

BF460294|BF460294|074A05 Mature tuber

AY007545|AY007545|Brassica napus

AC087544|AC087544|Oryza sativa

AB041503|AB041503|Populus nigra

35

The invention furthermore relates to modifying ELS genes or gene products or functional equivalents thereof which are for example derived from at least two different genes in the 40 Arabidonsis genome. They show high homology on protein level with the corresponding transmembrane RKS gene products.

However, they lack a transmembrane domain while they do

contain a signaling sequence at the N-terminal end. Therefore

these proteins are thought to be positioned within vesicles

5 within the plant cell or at the outside of the plasma membrane, within the cell wall of the plant cell. A number of homologs have been detected in other plant species, such as:

AF370543|AF370543|Arabidopsis thaliana

10 AF324989|AF324989|Arabidopsis thaliana AV520367|AV520367|Arabidopsis thaliana

AV553051 | AV553051 | Arabidopsis thaliana BF642233 | BF642233 | NF050C09IN1F1069

AW559436|AW559436|EST314484 DSIR Medicago truncata

15 BG456991 | BG456991 | NF099F02PL1F1025

AW622146|AW622146|EST312944 tomato

BF260895|BF260895|HVSMEf0023D15f Hordeum vulgare

BE322325 | BE322325 | NF022E12IN1F1088

BG414774|BG414774|HVSMEk0003K21f Hordeum vulgare

20 BE460627|BE460627|EST412046 tomato

BI204894|BI204894|EST522934 cTOS Lycopersicon esculentum BI205306|BI205306|BST523346 cTOS Lycopersicon esculentum BI204366|BI204366|EST522406 cTOS Lycopersicon esculentum AW443205|BST308135 tomato

25 AW031110|AW031110|EST274417 tomato

BI180080|BI180080|EST521025 cSTE Sclanum tuberosa

BF644761|BF644761|NF015A11EC1F1084

AV526127 | AV526127 | Arabidopsis thaliana

AV556193|AV556193|Arabidopsis thaliana

30 BE203316|BE203316|EST403338 KV1 Medicago truncatata.

AW649615|AW649615|EST328069 tomato

BE512465|BE512465|946071E06

BI204917|BI204917|EST522957 cTOS Lycopersicon esculentum

BG590749|BG590749|EST498591

35 BG648725|BG648725|EST510344 HOGA Medicago truncata

BG648619|BG648619|EST510238 HOGA Medicago truncata BG597757|BG597757|EST496435 cSTS Solanum tuberosa

AW221939|AW221939|EST298750 tomato

BE704836|BE704836|Sc01

40 BG1244091BG1244091EST470055 tomato

BF051954 BF051954 EST437120	tomato

BG320355|BG320355|Zm03_05h01_Zea mays

AV526624 | AV526624 | Arabidopsis thaliana AW933960 | AW933960 | EST359803 tomato

5 AW221278|AW221278|EST297747 tomato

BE405514 | BE405514 | WHE1212 CO1 F02ZS Wheat

BG314461|BG314461|WHE2495_A12_A23ZS Triticum

 $\underline{\mathtt{BF258673}} \hspace{0.1cm} |\hspace{0.1cm} \mathtt{BF258673}| \hspace{0.1cm} \mathtt{HVSMEf0016G01f} \hspace{0.2cm} \textit{Hordeum} \hspace{0.2cm} \textit{vulgare}$

BG262637|BG262637|WHE0938_E03_I06ZS Wheat

10 AW030188 | AW030188 | EST273443 tomato

BG653580|BG653580|sad76b11.yl Gm-c1051 Glycine max

BG319729|BG319729|Zm03_05h01_A Zm03_Zea mays BF053590|BF053590|EST438820 potato

BE454808|BE454808|HVSMEh0095C03f Hordeum vulgare

15 BI075801|BI075801|IP1 21 D05.b1 A002

BE367593|BE367593|PI1_9_F02.b1_A002 Sorghum bicolor 2e-074 BF260080|BF260080|HVSMEf0021A22f Hordeum vulgare

BF627921|BF627921|HVSMEb0006I23f Hordeum vulgare

BG598491|BG598491|EST503391 cSTS Solanum tuberosa

20 AW038168|AW038168|EST279825 tomato

BG343258|BG343258|HVSMEg0005D23f Hordeum vulgare

AW925684 | AW925684 | HVSMEg0005D23 Hordeum vulgare

BG416093|BG416093|HVSMEk0009L18f Hordeum vulgare

AW683370 | AW683370 | NF011C09LF1F1069

25 BE420108|BE420108|WWS020.C1R000101 ITEC WWS Wheat
AW350720|AW350720|GM210009A10F4 Gm-r1021 Glycine max

AW616564|AW616564|EST322975 L. Hirsutum trichome

AW011134|AW011134|ST17B03 Pine

BF630746|BF630746|HVSMEb0013N06f Hordeum vulgare

30 <u>AW926045</u>|AW926045|HVSMEg0006C10 Hordeum vulgare BE519800|BE519800|HV CEb0021E12f Hordeum vulgare

BG343657|BG343657|HVSMEg0006C10f Hordeum vulgare

BG933682|BG933682|OV1 16 C09.b1 A002

BE433368|BE433368|EST399897 tomato

35 AW219797|AW219797|EST302279 tomato

<u>BF629324</u>|BF629324|HVSMEb0010N06f Hordeum vulgare BE597128|BE597128|PI1 71 A07.g1 A002

AW220075|AW220075|EST302558 tomato

AW616639|AW616639|EST323050 L. Hirsutum trichome

40 BF645214|BF645214|NF032F11EC1F1094

AW924540|AW924540|WS1_70_H12.b1 A002

<u>A1775448</u>|A1775448|ES7256548 tomato <u>AM983360</u>|AW983360|HVSMEg0010F15f Hordeum vulgare <u>BE270171</u>|BE270171|GA_Eb0007B13f Cossypium arbor. <u>BE9319531</u>|BE919631|ES7423400 potato

5 ANO37836|ANO37836|EST279465 tomato BF008781|BF008781|sa79h09.yl Gm-ol064 Glycine max BF254651|BF254651|HVSMEf0004K051 Hordeum vulgare BE599797|BE599797|FII_79_R01.ql_A002

BE599026|BE599026|PI1_86_E03.g1_A002

10 R89998 R89998 116553 Lambda-PRL2 Arzhidopsis
BG641108 BG641108 BEST15-GO2.T3 ISUM4-TN Zea mays
AW307218 | NM307218 | Sf54c07.y1 Gm-c1009 Glycine max
AL496325 | AT496325 | sb05c09.y1 Gm-c1004 Glycine max
AU277703 | Zea mays

15 <u>AL375586</u> (CNS0616P|Medicago truncatula EST <u>AW350549</u> |AW350549|GM210009A10A12 Gm-r1021 Glycine max <u>BE125918</u> |BE125918|DG1_59_F02.bl_A002 <u>BF053901</u> |BF053901|KE7439131 potato <u>BE921389</u>|BE921389|ES7425266 potato

20 <u>BE597551</u>|BE597551|PII_71_A07.bl_ <u>BE360092</u>|BE360092|DG1_61_C09.bl_A002 <u>BE660084</u>|BE660084|491 GmaxSC Glycine max <u>AJ277702</u>|ZMA277702|Zea mays

25 The invention also relates to modifying SBP/SPL gene or products which represent a family of transcription factors with a bipartite nuclear localization signal (The SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE (SBP/SPL) gene family of Arabidopsis thaliana, Columbia ecotype). Upon activation (probably by RKS mediated phosphorylation, the bipartite nuclear localization signal becomes linear and available for the nuclear translocation of the protein. Within the plant nucleus, the transcription factor regulates transcription by interaction with specific promoter elements. In Arabidopsis 18thaliana, this family is represented by at least 16 different members (see following list). In many other plant species, we also identified members of this transcription factor family (See list on page 7).

Functional interaction between RKS and SBP proteins was shown by studies in transgenic tobacco plants in which SBP5 and RKSO were both overexpressed under the control of an enhanced 35S promoter (data not shown). At the tip of double overexpressing 5 plants, embryo structures appeared whereas in the SBP5 overexpressing plants alone or the RKSO overexpressing plants alone no phenotype was detectable at the root tips of transgenic tobacco plants. These results show that both RKS and SBP proteins are involved together in a signalling 10 cascade, resulting in the reprogramming of developmental fate of a determined meristem. (ref. dissertation: http://www.ub.uni-koeln.de/ediss/archiv/2001/11w1204.pdf; Plant Journal 1997: 12, 2 367-377; Mol. Gen. Genet. 1996: 250, 7-16; Gene 1999, 237, 91-104, Genes and Development 1997: 11, 15 616-628), Proc. Natl. Acad. Sci. USA 1998: 95, 10306-10311; The Plant Journal 2000: 22, 523-529; Science 1997: 278, 1963-1965; Plant Physiol. Biochem. 2000: 38, 789-796; Cell 1996: 84, 61-71; Annu. Rev. Plant Physiol. Plant Mol. Biol. 1999: 50, 505-537

20

	name	genetic code
	ATSPL1	At2g47070*
	ATSPL2	At5g43270
	ATSPL3	At2g33810*
25	ATSPL4	At1g53160*
	ATSPL5	At3g15270
	ATSPL6	At1g69170
	ATSPL7	At5g18830
	ATSPL8	At1g02065
30	ATSPL9	At2g42200*
	ATSPL10	At1g27370*
	ATSPL11	At1g27360*
	ATSPL12	At3g60030
	ATSPL13	At5g50570
35	ATSPL14	At1g20980
	ATSPL15	At3g57920
	ATSPL16	At1g76580

^{*} annotation in database not complete and/or correct

In many other plant species, we identified members of this transcription factor family, plant homologs of the Arabidopsis SBP/SPL proteins are for example:

- 5 AB023037|AB023037|Arabidopsis thaliana
 - BG789832|BG789832|sae56b07.yl Gm-c1051 Glycine max
 - BG123992|BG123992|EST469638 tomato
 - BG595750|BG595750|EST494428 cSTS Solanum tuberosum
 - AF370612|AF370612|Arabidopsis thaliana
- 10 BF728335|BF728335|1000060H02.x1 1000 Zea mays
 - X92079|AMSBP2|A.majus
 - AW331087|AW331087|707047A12.xl 707 Mixed adult... 128 zea mays
 - AJ011643|ATH011643|Arabidopsis thaliana
 - L34039|RICRMSOA|Orvza sativa
- 15 AJ011638|ATH011638|Arabidopsis thaliana
 - AJ011639|ATH011639|Arabidopsis thaliana
 - AJ132096|ATH132096|Arabidopsis thaliana
 - BF482644|BF482644|WHE2301-2304 A21 A212S Wheat
 - BF202242|BF202242|WHE0984_D01_G02ZS Wheat
- 20 BE057470|BE057470|sm58e10.yl Gm-c1028 Glycine max
 - AJ011628|ATH011628|Arabidopsis thaliana
 - AJ011629|ATH011629|Arabidopsis thaliana
 - AJ011617|2MA011617|Zea mays
- AJ011637|ATH011637|Arabidopsis thaliana
- 25 AJ011622|AMA011622|Antirrhinum majus
 - AJ011621|AMA011621|Antirrhinum majus
 - AJ011635|ATH011635|Arabidopsis thaliana
 - AJ011623|AMA011623|Antirrhinum majus
 - BF650908 | BF650908 | NF098D09EC1F1076
- 30 AJ242959 | ATH242959 | Arabidopsis thaliana
 - Y09427|ATSPL3|A.thaliana mRNA
 - AJ011633|ATH011633|Arabidopsis thaliana
 - AW691786 AW691786 NF044B06ST1F1000
 - BE058432|BE058432|sn16a06.yl Gm-c1016 Glycine max
- 35 AW728623|AW728623|GA Ea0017G06 Gossypium arbore.
 - BG442540|BG442540|GA Ea0017G06f Gossypium arbo.
 - AJ011626|ATH011626|Arabidopsis thaliana
 - AJ011625|ATH011625|Arabidopsis thaliana
 - AI993858|AI993858|701515182 A. thaliana
- 40 <u>BG593787</u>|BG593787|EST492465 cSTS Solanum tuberosum
 - BF634536|BF634536|NF060C08DT1F1065 Drought Medicago

PCT/NL2003/000524

BE806499|BE806499|ss59f10.yl Gm-c1062 Glycine max AW933950|AW933950|EST359793 tomato

AC008262|AC008262| Arabidopsis

B28493|B28493|T10A24TF TAMU Arabidopsis thaliana

5 AJ011644|ATH011644|Arabidopsis thaliana

AC018364|AC018364|Arabidopsis thaliana

AL092429|CNS00VLB|Arabidopsis thaliana

BE435668|BE435668|EST406746 tomato

BG0 97153 | BG0 97153 | EST4 61672 potato

10 <u>BE440574</u>|BE440574|sp47b09.yl Gm-c1043 Glycine max AI443033|AI443033|sa31a08.yl Gm-c1004 Glycine max

<u>U89496</u>|2MU89496|*Zea mays* liguleless1 AW433271|AW433271|sh54g07.v1 Gm-c1015 *Glycine max*

AW932595|AW932595|EST358438 tomato

15 AW096676|AW096676|EST289856 tomato

AJ011616|ZMA011616|Zea mays

AW036750|AW036750|EST252139 tomato

BF626329|BF626329|HVSMEa0018F24f Hordeum vulgare

AJ011614|ZMA011614|Zea mays

20 AJ011642|ATH011642|Arabidopsis thaliana

BE022435|BE022435|sm85h04.y1 Gm-cl015 Glycine max X92369|AMSPB1|A.majus

X32303 | AMSEBI | A.Majus

AC015450|AC015450|Arabidopsis thaliana AC079692|AC079692|Arabidopsis thaliana

25 AJ011632|ATH011632|Arabidopsis thaliana

AJ011631|ATH011631|Arabidopsis thaliana

BE455349|BE455349|HVSMEh0097E20f Hordeum vulgare

AJ242960|ATH242960|Arabidopsis thaliana

AJ011610 | ATH011610 | Arabidopsis thaliana

30 AJ132097 | ATH132097 | Arabidopsis thaliana

AL138658|ATT209|Arabidopsis thaliana

AJ011615|ZMA011615|Zea mays

BE499739|BE499739|WHE0975 Wheat

AW398794|AW398794|EST309294 L. pennellii

35 AJ011618 | ZMA011618 | Zea mays

AW747167|AW747167|WS1_66_F11.b1_

AJ011577|ATH011577|Arabidopsis thaliana AI992727|AI992727|701493410 A. thaliana

BE060783|BE060783|HVSMEg0013F15f Hordeum vulgare

DEGGOTOS | DEGGOTOS | AVSNEGGOTS FIST NOT GEGIN VGIGATE

40 <u>BE804992</u>|BE804992|ss34h10.y1 Gm-c1061 Glycine max BE325341|BE325341|NF120H09ST1F1009

AC007369|AC007369|Arabidopsis thaliana
AJ011619|ZMA011619|Zea mays
B109345|B1099345|IP1_37_H10.b1_A002
B1071295|B1071295|C054P790 Populus

5 A2920400|A2920400|1006019601.y2 1006 A2919034|A2919034|1006013602.x3 1006 BE805023|BE805023|ss35d09.y1 Cm-c1061 Glycine max
B6582086|BG582086|BS7483824 GVN Medicago truncata
AJ011609|ATH011609|Arabidopsis thaliana

10 BE023083|BE023083|sm90e08.y1 Cm-c1015 Glycine max

Furthermore, the invention relates to modifying NDR-NHL-genes or gene products. All proteins belonging to this family contain one (and sometimes even more than one) transmembrane 15 domain. Arabidopsis contains a large number of NDR-NHL genes, such as: aad21459, aaf18257, aac36175, k10d20 (position 40852-41619), aad21460, cab78082, aad21461, aad42003, aaf02134, aaf187656, aaf02133, cab43430, cab88990, cab80950, aad25632, aaf23842, a1163812, 20 f20d21-35, t13m11-12, f1e22-7, t23g18, f5d14-4266, t32f12-16, f11f19-11, f11f19-12, f11f19-13, t20p8-13, f12k2, f23h14, k10d20-44043, k10d20-12, t19f11-6, t19f11-5, t10d17-10, f22o6-150, f3d13-5, m3e9-80, t25p22-30, mhf15-4, mhf15-5, mrn17-4, mlf18-9, mgn6-11994, mjj3-9667, f14f18-60, At1q17620 F11A6, At5q11890 , At2q27080 , At5q36970 , 25 mlf18 , At1q65690 F1E22 , At4q01110 F2N1 , At2q35980 f11f19 , At4g01410 F3D13 , At1g54540 F2OD21 , At2g46300 t3f17 , At5g21130 , At3g11650 T19F11 , At5g06320 MHF15 , At5g06330 MHF15 , At2g01080 f15b18 , At2g35460 t32f12 , At2g27260 f12k2 , At2g35970 f11f19 , At5g53730 MGN6 , At5g22870 MRN17 , At4g09590 , At3g54200 , At1g08160 30 T6D22 , At5g22200 , At3g52470 , At2g35960 f11f19 , At3g52460 , At5g56050 MDA7, At3g20590 K10D20 , At1g61760 T13M11 , At3g20600 K10D20 , At1g13050 F3F19 , At3g11660 T19F11 , At3g44220 , At1g64450 F1N19 , At3g26350 F20C19 C , At4g05220 , At5g45320 K9E15 , At4q23930 , At4q13270 , At4q39740 , At1q45688 F2G19 W , At5q42860 35 MBD2 , At1g32270 F27G20 , At4g30660 , At2g45430 f4123 , At4g30650 , At1q69500 F10D13

and

40 ndr1, At2g27080; T20P8.13, At5g21130, At1g65690, At5g36970,

At1g54540, At5g06320, At5g11890, At1g17620, At3g11650, At2g22180, At5g22870, At2g35980, At2g46300, At4g05220, At2g35460, At2g46300, At4g05220, At2g35460, At2g47260, At4g01410, At5g22200, At1g61760, At3g52470, At5g53730, At4g01110, At2g35960, At3g52460, At4g0590, At2g35970, At3g26350, At3g11660, At3g44220, At1g08160, At2g01080, At5g06330, At5g56050, At3g20600, NDR1, At3g54200, At3g20590, At4g39740, At1g32270 syntaxin, putative, At1g13050, At5g45320, At3g20610, At4g26490, At5g42860, At1g45688,

10 NDR-NHL genes belong to a large family of which one of the first identified is the defence-associated gene HINI (Harpin-induced gene). HINI is transcriptionally induced by harpins and bacteria, that elicit hypersensitive responses in tobacco. It is thus believed that the genes of the invention also play arole in the hypersensitive reaction. Especially (see also chapter 8) since the genes of the invention bear relation to brassinoid-like responses and since brassinoid pathway compounds have been found to interact in this same defence system in plants. Other plant species also contain members of this large gene family, such as:

Plant homologs of the Arabidopsis NDR/NHL genes:

 25
 86582276| 86582276| 857484016 GVN Medicago truncata

 AV553539| Av553539| Arabidopsis thaliana

 AC059325| AC069325| Arabidopsis thaliana

 BC9526| AC069325| Arabidopsis thaliana

 BC953456| BC6583456| EST485208 GVN Medicago truncata

 BC967931| AW267833| EST485208 GVN Medicago truncata

 BE997791| BE997791| EST429516 GVSN Medicago truncata

 BE997991| BE997791| EST429516 GVSN Medicago truncata

 BC580928| BC580928| EST482657 GVN Medicago truncata

 AV544651| AV544651| Arabidopsis thaliana

 AV543762| AV543762| Arabidopsis thaliana

 AW559665| AW559665| EST314777 DSTR Medicago truncata

 BC581012| BG581012| EST482741 GVN Medicago truncata

 AV552664| AV54164| Arabidopsis thaliana

BE999881|BE999881|EST431604 GVSN Medicago truncata

40 AW0310981AW0310981EST274405 tomato

	WO 2004/007712
	11 AI998763 AI998763 701546833 A. thaliana
	AW219286 AW219286 EST301768 tomato
	BE124562 BE124562 EST393597 GVN Medicago truncata
	AV540371 AV540371 Arabidopsis thaliana
5	AV539549 AV539549 Arabidopsis thaliana
•	BG647432 BG647432 EST509051 HOGA Medicago truncata
	BE434210 BE434210 EST405288 tomato
	BG725849 BG725849 sae42g02.yl Gm-c1051 Glycine max
	APO03247 APO03247 Oryza sativa
10	BE348073 BE348073 spllall.yl Gm-cl042 Glycine max
	AW508383 AW508383 si40c06.yl Gm-r1030 Glycine max
	AI856504 AI856504 sb40b07.yl Gm-c1014 Glycine max
	BE556317 BE556317 sq01b07.y1 Gm-c1045 Glycine max
	AA713120 AA713120 32681 Arabidopsis
15	AV541531 AV541531 Arabidopsis thaliana
	A1894456 A1894456 EST263911 tomato
	AW704493 AW704493 sk53g11.yl Gm-c1019 Glycine max
	AW219298 AW219298 EST301780 tomato
	BF425685 BF425685 ss03c11.yl Gm-c1047 Glycine max
20	AV422557 AV422557 Lotus japonicus
	BE190816 BE190816 sn79a08.yl Gm-c1038 Glycine max
	BG580331 BG580331 EST482056 GVN Medicago truncata
	AV423251 AV423251 Lotus japonicus
	<u>A1896088</u> A1896088 EST265531 tomato
25	AV413427 AV413427 Lotus japonicus
	AV426656 AV426656 Lotus japonicus
	AV416256 AV416256 Lotus japonicus
	AL385732 CNS0690I Medicago truncatula
	AB016877 AB016877 Arabidopsis thaliana
30	AV419449 AV419449 Lotus japonicus
	AI486269 AI486269 EST244590 tomato
	AV411690 AV411690 Lotus japonicus
	AV419925 AV419925 Lotus japonicus
35	AV418222 AV418222 Lotus japonicus
50	AV409427 AV409427 Lotus japonicus
	ACOUSERT ACOUSERT Arabidopsis thaliana
	AV426716 AV426716 Lotus japonicus AV411791 AV411791 Lotus japonicus
	BG351730 BG351730 131E12 Mature tuber
40	BG046452 BG046452 saa54b12.y1 Gm-c1060 Glycine max
-10	DOG TO TO THE MAN

AI781777 | AI781777 | EST262656 tomato

BE451428|BE451428|EST402316 tomato AI7729441AI7729441EST254044 tomato AI895510|AI895510|EST264953 tomato AW030762|AW030762|EST274017 tomato 5 AW218859|AW218859|EST301341 tomato BE203936|BE203936|EST396612 KV0 Medicago truncata AV410289|AV410289|Lotus japonicus AW032019|AW032019|EST275473 tomato AW030868|AW030868|EST274158 tomato 10 AV421824|AV421824|Lotus japonicus BG646408|BG646408|EST508027 HOGA Medicago truncata AF325013|AF325013|Arabidopsis thaliana AC007234|AC007234|Arabidopsis thaliana AW217237|AW217237|EST295951 tomato 15 AC034257|AC034257|Arabidopsis thaliana AW625608|AW625608|EST319515 tomato AW031064|AW031064|EST274371 tomato AF370332|AF370332|Arabidopsis thaliana AB006700|AB006700|Arabidopsis thaliana 20 AW035467|AW035467|EST281205 tomato AL163812 | ATF14F18 | Arabidopsis thaliana AI896652|AI896652|EST266095 tomato AI730803|AI730803|BNLGHi7970 Cotton AW034775|AW034775|EST278811 tomato

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The invention provides the insight that RKS proteins or functional equivalents thereof play part in a signaling complex (herein also called the RKS signaling complex)

30 comprising molecules of RKS proteins, ELS (Extracellular Like SERK) proteins, NDR/NNL proteins and SBP/SFL (Squamosa Binding Protein) proteins, and the corresponding protein ligands (see for example table 3) whereby each of these proteins interplay or act in such a way that modifying genes, or modifying expression of genes, encoding ELS, RKS, NDR/NHL or SBP/SPL, proteins or said ligands may lead to functionally equivalent results (Figure 5. Two-hybrid interaction experiments have for example shown in vitro interaction between RKS 0 and NDRO/NHL28 and members of the SBP/SPL family. Here we show 40 that in vivo the individual components of this signaling

complex are regulating identical processes, as based on functional genomics on transgenic plants, overexpressing or co-suppressing single components or combinations of components in this transmembrane signalling complex. ELS gene products 5 are derived from at least two different genes in the Arabidopsis genome. They show high homology on protein level with the corresponding transmembrane RKS gene products.

However, they lack a transmembrane domain while they do contain a signalling sequence at the N-terminal end. Therefore these proteins are thought to be positioned within vesicles within the plant cell or at the outside of the plasma membrane, within the cell wall of the plant cell. A number of homologues have been detected in other plant species (see list on page 3). ELS proteins are involved in the heterodimerizing 15 complex with the RKS transmembrane receptor at the outer membrane site. ELS molecules are either in competition or collaboration with RKS molecules involved in the high affinity binding of the ligand. The signal transmitted from the ligand onto the RKS proteins is then transporter over the membrane 20 towards the N-terminal site of RKS protein, located on the other site of the membrane. The activation stage of the RKS molecule is changed, as a result of transphosphorylation by dimerizing receptor kinase dimerizing partners. Subsequently the signal is transmitted to other proteins, one family of such proteins is defined as the SBP/SPL family of transcription factors, the other family of proteins is represented by the NDR/NHL members.

The different obvious phenotypes created by modifying the 30 RKS gene products could be effected by one process regulating all different effects in transgenic plants.

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All the phenotypes observed can be effected by the process of brassinosteroid perception. In chapter 1, RKS genes are clearly involved in plant size and organ size. Loss of RKS expression results in a dwarf phenotype, similar as observed with brassinosteroid synthesis mutants. It was already known in literature that the phenotypes observed from modifying the

RKS genes are also observed when modifying the brassinosteroid pathway genes and/or their regulation, thereby altering the amount and nature of the brassinosteroids in plants.
Literature which describes the phenotypic effects of modifying 5 teh brassionosteroid pathway can, amogst others, be found in: Plant Journal 26: 573-582 2001; Plant Journal 1996 9(5) 701-713, genetic evidence for an essential role of brassinosteroids in plant development; J. Cell Biochem Suppl. 21a 479 (1995); Mandava 1988 Plant growth-promoting 10 brassinosteroids, Ann. Rev. Plant physiol. Plant Mol. Biol. 39 23-52; Plant Physiol 1994 104: 505-513; Cell 85 (1996) 171-182; Clouse et al. 1993 J. Plant Growth Regul. 12 61-66; Clouse and Sasse (1998) Annu. Rev. Plant Physiol. Plant Mol. Biol 49 427-451; Sasse, Steroidal Plant Hormones. Springer-15 Verlag Tokyo pp 137-161 (1999).

It is thus believed, without being bound to any theory, that modification of the RRS genes will result in a modification of the brassinosteroid pathway, thereby giving the various phenotypes that are shown below.

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"Functionally equivalent" as used herein is not only used to identify the functional equivalence of otherwise not so homologous genes encoding ELS, RKS, NDR/NHL or SBP/SPL proteins, but also means an equivalent gene or gene product of genes encoding ELS, RKS, NDR/NHL or SBP/SPL proteins in Arabidopsis Thaliana, e.g. identifying a homologue found in nature in other plants or a homologue comprising a deliberate nucleic acid modification, such as a deletion, truncation, insertion, or deliberate codon substitution which may be made on 30 the basis of similarity in polarity, charge, solubility, hydrophobicity, and/or the amphipathic nature of the residues as long as the biological activity of the polypeptide is retained. Homology is generally over at least 50% of the full-length of the relevant sequence shown herein. As is well-understood, 35 homology at the amino acid level is generally in terms of amino acid similarity or identity. Similarity allows for "conservative variation", i. e. substitution of one

hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Deliberate amino 5 acid substitution may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, and/or the amphipathic nature of the residues as long as the biological activity of the polypeptide is retained. In a preferred embodiment, all percentage homologies referred to herein refer 10 to percentage sequence identity, e.g. percent (%) amino acid sequence identity with respect to a particular reference sequence can be the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, without considering any conservative substitutions as part of the sequence identity. Amino acid similarity or identity can be determined by genetic programs known in the art.

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'Plant cell', as used herein, amongst others comprises seeds, suspension cultures, embryos, meristematic regions, callous tissues, protoplasts, leaves, roots, shoots, bulbs, gametophytes, sporophytes, pollen and microspores. A target plant to be modified according to the invention may be selected 25 from any monocotyledonous or dicotyledonous plant species, such as for example ornamental plants, vegetables, arable crops etc. 'Dicotyledoneae' form one of the two divisions of the flowering plants or angiospermae in which the embryo has two or more free or fused cotyledons. 'Monocotyledoneae' form one of the two divisions of the flowering plants or angiospermae in which the embryo has one cotyledon. 'Angiospermae' or flowering plants are seed plants characterized by flowers as specialized organs of plant reproduction and by carpels covering the ovaries. Also included are gymnospermae. Gymnospermae are seed plants 35 characterized by strobili as specialized organs for plant reproduction and by naked sporophylls bearing the male or female reproductive organs, for example woody plants. 'Ornamental'

plants are plants that are primarily in cultivation for their habitus, special shape, (flower, foliage or otherwise) colour or other characteristics which contribute to human well being indoor as cut flowers or pot plants or outdoors in the man made 5 landscape, for example bulbous plant species like Tulips, Freesia, Narcissus, Hyacinthus etc. 'Wegetables' are plants that are purposely selected or bred for human consumption of foliage, tubers, stems, fruits, flowers or parts of them and that may need an intensive cultivation regime. 'Arable crops' are 10 generally purposely bred or selected for human objectivity's (ranging from direct or indirect consumption, feed or industrial applications such as fibers) for example soybean, sunflower, corn, peanut, maize, wheat, cotton, safflower and rapeseed.

The invention provides a method for modulating a developmental 15 pathway of a plant comprising modifying a gene encoding for a gene product or protein belonging to a developmental cascade or signaling complex comprising modifying at least one gene encoding a gene product belonging to the complex of RKS proteins, ELS proteins, NDR/NHL proteins, SBP/SPL proteins and 20 ligand proteins. In one embodiment, the invention provides a method for modulating or modifying organ size. Plant or plant organ size is determined by both cell elongation and cell division rate. Modifying either one or both processes results in a change in final organ size. Increasing the level of 25 specific members of the family of RKS genes results in an increase in organ size, growth rate and yield. Modulating plant growth, organ size and yield of plant organs is the most important process to be optimized in plant performance. Here we show that modulating the level of members of the family of the RKS signaling complex with a method according to the 30 invention is sufficient to modulate these processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein 35 said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKS10 gene or functional equivalent thereof. Inactivation of endogenous RKS gene product results in a decrease in plant growth,

- 5 proving that the normal function of these endogenous RKS gene products is the regulation of growth and organ size. Use of a method according to invention for elevation of the levels of the regulating of the RKS signaling complex in plant cells is provided in order to increase for example the size of plant
- organs, the growth rate, the yield of harvested crop, the yield of total plant material or the total plant size.

 Decreasing the levels of endogenous RKS gene product is provided in order to decrease the size of plant organs, the growth rate, or the total plant size.
- 15 In another embodiment, the invention relates to cell division. The mitotic cell cycle in eukaryotes determines the total number of cells within the organism and the number of cells within individual organs. The links between cell proliferation, cell differentiation and cell-cycle machinery 20 are of primary importance for eukaryotes, and regulation of
- these processes allows modifications during every single stage of development. Here we show that modulating the level of members of the family of the RKS signaling complex is sufficient to modulate these processes. The invention provides
- 25 herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein. ELS protein, NDR/NHL protein, SBF/SFL protein and
- 30 RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKS10 gene or functional equivalent Herewith the invention provides a method for modulating the number of cells to be formed within an
- 35 eukaryotic organism as a whole or for modulating the cell number within individual organs is, which of primary importance in modulating plant developmental processes,

especially of arable plants. Here we show that members of the RKS signaling complex are able to regulate the number of cellular divisions, thereby regulating the total number of cells within the oranism or different organs.

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In a further embodiment, the invention relates to the regeneration of apical meristem. Modification the levels of different RKS and ELS genes within plants allows the initiation and / or outgrowth of apical meristems, resulting in the formation of large numbers of plantlets from a single source. A number of gene products that is able to increase the regeneration potential of plants is known already. Examples of these are KNAT1, cvcD3, CUC2 and IPT. Here we show that modulation of the endogenous levels of RKS genes results in the formation of new shoots and plantlets in different plant species like Nicotiana tabacum and Arabidopsis thaliana. Herewith the invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating apical meristem formation, in particular wherein said gene comprises an ELS1, RKS0, RKS3, RKS4, RKS8 or RKS10 gene or functional equivalent thereof. A direct application of such a method according to the invention is the stable or transient expression of RKS and ELS genes or gene products in order to initiate vegetative reproduction. Regeneration can be induced after overexpression of for example RKSO and ELS1; or 30 by co-suppression of for example the endogenous RKS3, RKS4, RKS8 or RKS10 genes. Overexpression or co-suppression of these RKS and ELS gene products can be either transient, or stable by integration of the corresponding expression casettes in the plant genome. A further example of essentially identical 35 functions for for example ELS1 and RKS0 overexpressing plants is for example shown in the detailed description, example 3, where both transgenic constructs are able to induce the

regeneration capacity of in vitro cultured Arabidopsis callus. Another example comprises functional interaction between RKS and SBP proteins which was shown by studies in transgenic tobacco plants in which SBP5 and RKSO were both overexpressed under the control of an enhanced 35S promoter. At the tip of double overexpressing plants, embryostructures appeared whereas in the SBP5 overexpressing plants alone or the RKSO overexpressing plants alone no phenotype was detectable at the root tips of transgenic tobacco plants. These results show that both RKS and SBP proteins are involved together in a signaling cascade, resulting in the reprogramming of developmental fate of a determined meristem. Furthermore, it is herein also shown that several RKS genes are able to regulate proper identity and development of meristems and primordia. The invention for example also relates to fasciation, Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the shoot apical meristem results in an excess number 20 of cellular divisions, giving rise to excess numbers of primordia formed or to stems in which the number of cells is increased. The invention herewith provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a 25 signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating fasciation, in particular wherein said gene comprises an RKSO, RKS3, RKS8 or RKS10 gene or functional 30 equivalent thereof. Here we for example show that modulation of the levels of RKS gene products in plants like Arabidopsis thaliana can result in fasciated stems. A direct application as provided herein is the regulated formation of fasciation in plant species in which such a trait is desired like 35 ornamental plants. Regulation of the initiation and extent of fasciation, either by placing the responsible RKS encoding DNA sequences under the control of stage or tissue specific

promoters, constitutive promoters or inducible promoters results in plants with localized or consitutive fasciation of stem tissue. Another application is modulating the number of primordia by regulation of the process of fasciation. An 5 example is provided by for example sprouts, in which an increased number of primordia will result in an increased numbers of sprouts to be harvested. Fasciation can also result in a strong modification in the structural architecture of the inflorescence, resulting in a terminal group of flowers 10 resembling the *Umbelliferae</code> type.

Identical phenotypes can be observed when transgenic plants are produced that contain the NHL10 cDNA under control of an enhanced 35S promoter. The resulting phenotype of the resulting flowers show that flower organ primordia are 15 switched in identity, similar as observed for RKS10 and RKS13. These meristematic identity switches are normally never observed in Arabidopsis and the fact that two different classes of genes are able to display the same phenotypes in transgenic plants is a clear indication for a process in which 20 both members of the RKS and the NDR/NHL families are involved. The invention also relates to root development. Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the root apical 25 meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to roots in which the number of cells is increased. Adaptation to soil conditions is possible by regulation of root development of plants. Here we describe several processes in root development that can me manipulated by modification of the levels of RKS signaling complex within the root. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein 35 belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating root development, in particular

wherein said gene comprises an ELS1, ELS2, RKS1, RKS3, RKS4, RKS6. RKS8 or RKS10 gene or functional equivalent thereof. Root length, a result by either root cells proliferation or elongation, can for example be increased by overexpression of 5 for example RKS3, RKS4, RKS6 and ELS2, or inactivation of the endogenous RKS10 gene product. Root length can also be decreased by decreasing of endogenous RKS1 levels or by strong overexpression of RKS10. The initiation of lateral roots is also regulated by RKS gene products. Overexpression of for 10 example RKS10 can result in a strong increase in the initiation and outgrowth of lateral roots. Co-suppression of RKS1 also resulted in the initiation and outgrowth of large numbers of lateral roots. Root hair formation and elongation is important in determining the total contact surface between 15 plant and soil. A strong increase of root hair length (elongation) can be obtained by overexpression of ELS1 and RKS3 gene products. As the roots of terrestrial plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant hormones, interaction with 20 the rhizosphere and storage functions, increasing or decreasing root length, for example for flexible adaptations to different water levels, can be manipulated by overexpressing or cosuppressing RKS and / or ELS gene products. Modulation of the total contact surface between 25 plant cells and the outside environment can be manipulated by regulation lateral root formation (increased by RKS10 overexpression and co-suppression of RKS1). Finally the contact surface between plant cells and the soil can be influenced by modulation of the number of root hairs formed or 30 the elongation of the root hairs, as mediated by ELS1 and RKS3

In a further embodiment, the invention relates to apical meristem identity. All parts of the plant above the ground are generally the result on one apical shoot meristem that has 35 been initiated early at embryogenesis and that gives rise to all apical organs. This development of a single meristem into complex tissue and repeated patterns is the result of tissue

22 and stage-dependent differentiation processes within the meristems and its resulting offspring cells. The control of meristem formation, meristem identity and meristem differentiation is therefore an important tool in regulating 5 plant architecture and development. Here we present evidence the function of RKS and ELS gene products in regulation of the meristem identity and the formation and outgrowth of new apical meristems. The invention provides a method for modulating a developmental pathway of a plant or plant cell 10 comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating meristem identity, in particular wherein said gene 15 comprises an ELS1, RKS8, RKS10 or RKS13 gene or functional equivalent thereof. Introduction of for example the RKS10 gene product or an other member of the RKS signaling complex under the control of a tissue and / or stage specific promoter as provided herein allows localized and time regulated increases

in the levels of gene product. For example the meristematic identity in a determined meristem might thereby be switched back into an undetermined meristem, thereby changing for example a terminal flower into an undetermined generative meristem.

25 Another application might be found in changing the meristematic identity at an early time point, during early vegetative growth, thereby switching the vegetative meristem into a generative meristem, allowing early flowering. Modulation of meristem identity in terminal primordia, like 30 for example as shown in Figure 30, where flower organ primordia are converted into terminal flower primordia, allows the formation of completely new types of flowers and fused fruitstructures. Constitutive overexpression of RKS gene products results in plants with many apical meristems, as can

35 clearly been seen in Figure 29, where RKS10 overexpression results in an extremely bushy phenotype.

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In another embodiment, the invention relates to male sterility. Male sterility is a highly desired trait in many plant species. For example, manipulation of pollen development is crucial for F1 hybrid seed production, to reduce labour

- 5 costs and for the production of low-environmental impact genetically engineered crops. In order to produce hybrid seed from inbred plant lines, the male organs are removed from each flower, and pollen from another parent is applied manually to produce the hybrid seed. This labour-intensive method is used 0 with a number of vegetables (e.g. hybrid tomatoes) and with
 - many ornamental plants. Transgenic approaches, in which one or more
 - introduced gene products interfere with normal pollen initiation and development is therefore highly desired.
- 15 Especially when the number of revertants (growing normal pollen) is extremely low.
 - Male sterility in plants is a desired trait that has been shown already in many plant species as a result of the inactivation of expression of a number of genes essential for
- 20 proper stamen development, mitotic divisions in the pollen stem cells, or male gametogenesis. A method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling
- 25 complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating pollen development, in particular wherein said gene comprises an ELS2 or RKS10 gene or functional equivalent thereof.
- 30 Here we present data that show that overexpression of gene products, like transmembrane receptor kinases (RKS) and extracellular proteins (ELS) can also result in the formation of male sterility. The ability to induce male sterility by overexpressing specific genes as provided herein allows the 35 opportunity to produce transgenic overexpressing plants in
 - b opportunity to produce transgenic overexpressing plants in which the pollen development is inhibited. Stable single copy homozygous integration of such overexpressing traits into the

plant genome will render such plants completely sterile, making them excellent material for the production of F1 hybrid seed. Furthermore, the combined integration of a male sterility inducing overexpressing gene coupled directly with

- 5 another desired transgene result in transgenic plants which
 are unable to produce transgenic seed, making these transgenic
 plants excellent material for outside growth without problems
 affecting transgenic pollen spreading throughout the
 environment, thereby eliminating possible crosses
- 10 with wild plant species or other non-transgenic crops. The combination of a desired transgene flanked on both sites by different male-sterility inducing overexpressing genes would decrease the frequency of pollen formation to an extremely low level. An example is an overexpressing construct of RKS10
- 15 at the 5'end of integrated DNA fragment, the desired transgene expression cassette in the middle and at the 3'end of the integrated DNA the ELS2 overexpressing construct. This complete DNA fragment is integrated into the genome by conventional techniques, like particle bombardment,
- 20 Agrobacterium transformation etc. Another possible application concerns the modification of pollen in ornamental plant species like lily, where the release of pollen from cut flowers can be avoided by making transgenic plants in which pollen development is initiated by release from the stamen is
- overexpressing for example ELS2, resulting in partial pollen development). Hereby the ornamental value of the stamen with pollen is not lost, but release of pollen is inhibited.

prevented (a desired trait that can be obtained by

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Furthermore, surprisingly we observe that NDR NHL gene
30 products share homology with the family of syntaxins, involved
in vesicle transport, positioning of cell wall formation and
cytokinesis.

PCT/NL2003/000524

Table 1

Homology between members of the syntaxin family and the NDR NHL family

25

5 NHL10= At2q35980

maaeqplnga fygpavpppa pkgyyrrghg rgcgccllel fvkviisliv ilgvaalifw livppraikf hvtdasltrf dhtspdnilr ynlaltvpvr npnkriglyy drieahayye gkrfstitlt pfyqghkntt vltptfqgqn lvifnagqsr tlnaeriagv ynielkfrlr vrfklgdlkf rrikpkvdod dirlpletsn gttttstvfp ikodfdf (SEQID.NO.1)

10 Atlg32270 syntaxin,

> MYRSNDVKFQ VYDAELTHFD LESNNNLQYS LSLNLSIRNS KSSIGIHYDR FEATVYYMNQ RLGAYPMPLF YLGSKNTHLL RALFEGGTLV LLKGNREKKF EDOGNTGYVR IDVKLSINFR VWYLHLVYMP MKPVYRCHLK IPLALGSSNS TGGKKMMLI GQLVKDTSAN LREASETDHR RNYACKKKIA DAKLAKDFRA ALKEDOKAOH ITVERETSYI PPDEKGSFSS SEVDIGYDRS

15 RDVAQSKKIA DAKLAKDERA ALKERQKAQH ITVERRTSYI PEDEKGSFSS SEVDIGYDRS

CROKVIMESE RGEIVILIDNE ISLNEARIEA REGGIGEVKH QUISEVMEMFK DLAVMVDHQG

TIDDIDEKID NLRSARAGK SHLVKASNYQ GSNSSLIFSC SLLIFFFISG DLCRCVCVGS

ENPRLNPTRR KAMCEEDDE QRKKQQKKKT MSEKRRREEK KVNKFNGFVF CVLGHK* (SEQID NO: 2)

20

Below the homology is shown between NHL10 (Upper line) and a syntaxin protein. (bottom line). The identical amino acids are shown in the middle line.

25

IVRPRAIKFHVTDASLTRFDHTSPDNILRYNLALTVPVRNPNKRIGLYYDRIEAHAYYEG
VR KF V DA LT FD S N L Y L L RN IG YDR EA YY
MVRSNDUKFOVYDAELTHFDLESNNN-LOYSLSLNLSIRNSKSSIGIHYDRFEATVYYMN

30

KRFSTITLTPFYQGHKNTTVLTPFFQGQNLVIFNAGQSRTLNAERISGVYNIEIKFRLRV
R FY G KNT L F GQ LV GVY I K
ORLGAVPMPLFYLGSKNTMLLRALFEGOTLVLLKGNERKKFEDDQKTGVYRIDVKLSINF

35 RFKLGDLKFRRIKPKVDCDDLRLPLSTSNGTTT

R L KP V C L PL T RVMVLHLVTWPMKPVVRCH-LKIPLALGSSNST

That syntaxins and NDR/NHL genes share large homology becomes even more clear when performing a database search using the following site:

http://mips.gsf.de/proj/thal/db/search/search frame.html

5 searching for homologous sequences with the sequence At1g32270

gene Code: predicted function:

	gene Code:	predicted function:
	At1g32270 syntaxin, putative	Syntaxin
10	At5g46860 syntaxin related protein	Syntaxin
	AtVam3p (gb AAC49823.1)	
	At4g17730 syntaxin	Syntaxin
	At5g16830 syntaxin homologue	Syntaxin
	At3g11650 unknown protein	Putative syntaxin
15	At2g35460 similar to harpin-induced prot	ein Putative syntaxin
	At5g06320 harpin-induced protein-like	Putative syntaxin
	At2g35980 similar to harpin-induced prot	ein Putative syntaxin
	At1g65690 hypothetical protein	NDR HNL
	At4g05220 putative protein	Putative syntaxin
20	At3g05710 putative syntaxin protein	Syntaxin
	AtsNAP33	
	At2g27080 unknown protein	NDR HNL
	At3g52470 putative protein	Putative syntaxin
	At1g61760 hypothetical protein	Putative syntaxin
25	At5g21130 putative protein	NDR HNL
	At3g52400 syntaxin-like protein synt4	Syntaxin
	At2g35960 putative harpin-induced protei	n Putative syntaxin
	At5g06330 harpin-induced protein-like	Putative syntaxin
	At5g26980 tSNARE	Syntaxin
30	At5g36970 putative protein	Putative syntaxin
	At3g44220 putative protein	Putative syntaxin
	At3g03800 s-syntaxin-like protein	Syntaxin
	At2g35970 putative harpin-induced protei	n Putative syntaxin
	At4g09590 putative protein	Putative syntaxin
35	At4g23930 putative protein	
	At1g61290 similar to syntaxin-related pr	otein Syntaxin
	At3g11660 unknown protein	Putative syntaxin
	At1g54540 hypothetical protein	Putative syntaxin
	At3g24350 syntaxin-like protein	Syntaxin
40	At5g22200 NDR1/HIN1-like	NDR HNL

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			***	27		C 1714L2003/000.
		At1g11250	syntaxin-related prot		Syntaxin	
		At5g53880				
		At3g11820	putative syntaxin		Syntaxin	
		At3g54200			Putative	syntaxin
	5	At5g05760	t-SNARE SED5		Syntaxin	
		At5g53730			Putative	syntaxin
		At4g03330	SYR1-like syntaxin 1		Syntaxin	
		At3g47910				
		At5g08080	syntaxin-like proteir	1	Syntaxin	
1	.0	At5g11890			Putative	syntaxin
		At1g17620			Putative	syntaxin
		At2g22180			Putative	syntaxin
		At5g22870			Putative	syntaxin
		At2g46300			Putative	syntaxin
1	.5	At2g27260			Putative	syntaxin
		At4g01410			Putative	syntaxin
		At5g22200			Putative	syntaxin
		At4g01110			Putative	syntaxin
		At3g52460			Putative	syntaxin
2	0	At3g26350				syntaxin
		At1g08160				syntaxin
		At2g01080	•		Putative	_
		At5g56050			Putative	_
_		At3g20600			Putative	
2		At3g20590			Putative	•
		At4g39740			Putative	-
		At1g32270			Putative	-
		At1g13050			Putative	
		At5g45320			Putative	-
3		At3g20610			Putative	-
		At4g26490			Putative	-
		At5942860			Putative	-
		At1g45688			Putative	-
		At4g26820			Putative	syntaxin
3	5					

This observation provides the explanation for understanding
40 the mechanism by which the RKS / NDR-NHL complex functions.

Cell wall immobilized RKS gene products (containing the

extensin-like extracellular domain) respond to a local ligand signal, in combination with the heterodimerizing ELS protein (s) either as homodimers, as RKS heterodimers or in combination with the heterodimerizing ELS protein(s).

- 5 Predicted ligands for the RRS / ELS receptor binding consist of peptide ligands (based on the LRR ligand binding domain of this class of receptors). These ligands are normally produced as a pre pro protein. The N-terminal signal sequence is removed by the transport through the Golgi system and
- allows modification of the ligand at this stage (e.g. glycosylation). The ligands can then be secreted after which further processing is possible (e.c. proteolytic cleavage, removal of sugar groups etc.) The resulting peptide, possible as a monomer or a (hetero)dimerizing molecule binds the
- 15 transmembrane receptor complex with high affinity, resulting in transmission of the signal from the ligand through the transmembrane receptor component towards the other site of the membrane.

One class of ligands interacting with the RKS and / or ELS 20 receptors consists of the family of pre(pro)proteins shown hereunder in table 3.

Table 3 Ligands within the RKS signaling complex (herein also called RKS/ELS ligand proteins)

- For each ligand (A to N) the genomic structure before splicing and processing 5'- towards 3' is given. Exons are indicated in large letters; introns and surrounding sequences (including leader 5'-and trailer sequences 3'-) are indicated in small letters. Beneath each DNA sequence the main oacid sequence of the pre-pro-
- peptide is given. The first line represents the signal sequence

 10 The second (set of) lines represents the pro-peptide.
 - The last line represents the conserved Cysteine motif.

A. At1g22690

- 15 1 attamacqcc amacactaca tetgtgtttt cgaacaatat tgcgtctgcg tttccttcat 61 ctatetetet cagtoteaca atotetgaac taagagacag etgtaaacta teattaagac 121 ataaactacc aaagtatcaa gctaatgtaa aaattactct catttccacg taacaaattg 181 agttagetta agatattagt gaaactaggt ttgaatttte ttettettet tecatgeate 241 ctccgaaaaa agggaaccaa tcaaaactgt ttgcatatca aactccaaca ctttacagca 20 301 aatgcaatct ataatctgtg atttatccaa taaaaacctg tgatttatgt ttggctccag 361 cgatgaaagt ctatgcatgt gatctctatc caacatgagt aattgttcag aaaataaaaa 421 gtagotgasa tgtatotata taaagaatos tocacaagta ctattttcac acactactto 481 asaatcacta ctcaagaaat ATGAAGAAGA TGAATGTGGT GGCTTTTGTT ACGCTGATCA 25 541 TCTCTTTTCT TCTGCTTTCT CAGgtasact gttasaacca ttttcaagac taccttttct 601 ctatttcaga caaaccaaag taaaacaatg aaaaatctct ctggtctttc atagGTACTT 661 GCAGAGTTGT CATCATCCAG CAACAATGAA ACTTCCTCTG TTTCTCAGgt aagagtgata 721 caaaaacata ctaaacaaac tttcaagaga gtaatatata aggaaatgtt ggcttctttt
 781 ttttgttgct aatcagACGA ATGACGAGAA CCAAACTGCG GGGTTTAAGA GAACATACCA 30 841 CCATCGTCCA AGAATCAgtt agtotactct ttcaacactc taattccttt gttctaagta 901 tttttttgc cccccacaac ctttttttta ttasatgagc caatttttat agATTGTGGG 961 CATGCATGCG CAAGGAGATG CAGTAAGACA TCGAGGAAGA AAGTTTGTCA CAGAGCCTGT 1021 GGAAGTTGTT GTGCCAAGTG TCAGTGTGTG CCGCCGGGAA CCTCCGGCAA CACAGCATCA 1081 TGTCCTTGCT ACGCCAGTAT CCGTACACAT GGCAATAAAC TCAAATGTCC TTAAsagact 35 1141 totcatttot caactatagt otcatottot gattatgttt ottottttgt tatgttgcat 1201 ctgtgatgtg tgagcttatt attatgttga ttgttgacat aattcaacta tataatttgt 1261 atcgattccg aataataaga tgagtgattt tattggctat taagtttttt ttttttttt 1321 ttgggcacaa tggctattaa gttttaaaca tctgatttta ttggttacaa asaacaacaa 1381 agtttcattt tcatattaac acaaaatctc catacatatt accaaaccaa aaaaatacac 40 1441 aagggggaga gagaccaacg gttcttggtt cagagtttgc atcttgtttg agccgtcacc 1501 gtttcttaga cttaacagca acaacacctt tataagctt cacqcatcc ttcaacqcat 1561 ctcgccgagg ccgagccacc ttattgtttg gatcaaacaa caaaacttct tcaacqcat 1621 tcaatgccaa aggc (SEQ ID NO: 3)
- 45 MKKMNVVAFVTLIISFLLLSQVLA

ELSSSSNNETSSVSOTNDENOTAAFKRTYHHRPRIN

CGHACARRCSKTSRKKVCHRACGSCCAKCQCVPPGTSGNTASCPCYASIRTHGNKLKCP* (SEQ ID NO; 4)

50

B. At1g74670

1 gaasaaaaga agasaagata atggtccgta ttaatatagt tgaasacttg asactacttt 5 61 ttaqtttqta tataatacaq tagactaggg atccaqttqa qtttctttct ttattttgag 121 tttgtgttta tgtttgattt tacgttttta tatgtaaata agatatttta cgaattatgg 181 ttttatttgg gtagaagttg tagaatgact taaacaatca agtggcagaa tgagatatat 241 aaagtaatat aatatatgta ccgttattaa cttattgtac atgtgaatga ggaagcttac 301 acacacacac cttctataaa tagctgacaa aactggttgt tacacacaaa acattcataa 10 361 attiticasa giasgasta agagittis tacagicita etetetaca attitice 421 teteteaga getagienis GCLANGTIA TARCITICTT TOTITICATO ACATITIA 481 TECRITICOT TOTICTACT AUTOCAMAS ARCITICATO CONTOCASA ACTICAGO 541 tttattttt ggtaasatag aaagtgtaag ttttataatt cattcaatty tttttgcctt 601 tocotttota tttattgota taaatotaat accogcotta aaatttgttt tgaaattaaa 661 cagrarggac Caggaagrot Gaarcatac Cogtaagtaaa aacttottot tottitatga 15 721 atcttgtttc ttattatata tcasatassa actcgattat catgattgca gAATGTGGAG 781 GACAATGCAC AAGGAGATGT AGCAACACAA AGTATCATAA GCCATGCATG TTCTTCTGCC 841 AAAAGTGTTG TGCTAAATGC CTTTGTGTCC CTCCAGGCAC GTACGGCAAC AAACAAGTGT 901 GTCCTTGTTA CAACAACTGG AAGACTCAAC AAGGTGGACC AAAATGTCCA TAAacaaaaa 20 961 cattyggaga gaaacoccaa totgettoct attitatta attattoca gatagetttt 1021 gttgtogtga tggttaaatt atagtgtttt tgcaggtatc attitatcatc gataaacaat 1001 atcatataaa atcttctatg tttctttcac gttttgttt tttttgta gtcatacac 1141 gaaatgtgta tggaccttct aattaggaat atataaaatt ttattatta attagataat 1201 ctttcgtata gttamaattc caaggattac ttttgattcg tttgggacaa tctattttat 25 1261 attttacttt ctaagtttgt ataactatat cttaaaagtg ttagacagag toctaatgat 1321 tttagtataa ttgttactat ttagttacgc ttcgaaaatt tggaactttt ccaaagtggt 1381 ctatatoaat tigaticact autotycyct toottotagt tittitacaat taiggagatt 1441 tttcgacgat gat (SEQ ID NO: 5)

30

35

MAKLITSFLLLTILFTFVCLTMS

KEAEYHPESYGPGSLKSYQ

CGGQCTRRCSNTKYHKPCMFFCQKCCAKCLCVPPGTYGNKQVCPCYNNWKTQQGGPKCP * (SEQ ID NO: 6)

C. At1g75750

1 cacaactttt atacgcacca ccaaccgacc cattttgaaa aagagaaaat aaaccacaaa 5 61 aacacacata aataatatgo tgataacaat gtottaaaaa totatttaco atttotagta 121 atcastatot attycasasa atatttataa gaatacasst gasasatyat aasstacass 181 tgatttctca attacctama mamatatama atgtcttact ttattttcag ccactgttgg 241 saagtacttg caatcatatc gtattttgaa ttatasaact cagaaacaat tattttccct 10 361 taggategat tatecateea aaggagtact atamatagaa ceeteeaagt teteattagg 421 acacacaca taaaaccac ttatcatta caytotgatt tgagctaagt tctctatca 481 taaactctc ttggagaatc ATGGCTATT CAAAAGCTCT TATCGCTTCT CTTCTCATAT 541 CTCTTCTTGT TCTCCAACTC GTCCAGGCTG ATGTCGtacg tctttttcat cacaaactaa 601 ttatactcaa tataatactt atgitttcaa aaacatatti ctcacatgit acaacaatat 661 tcttgcagGa AAACTCACAG AAGAAAAATG GTTACGCAAA GAAGATCGgt aattatatga 15 721 tttttattaa acctaacgtt aaatttagag tgagattaat aatctgtgtt tttctttctt
781 gtatatatag ATTGTGGAG TGCGTGTGTA GCACGGTGCA GGCTTTCGAG GAGGCCGAGG 841 CTGTGTCACA GAGCGTGCGG GACTTGCTGC TACAGGTGCA ACTGTGTGCC TCCGGGTACG 901 TACGGAAACT ACGACAAGTG CCAGTGCTAC GCTAGCCTCA CCACCCACGG TGGACGCCGC 20 961 AAGTGCCCAT AAgaagaaac aaagctctta attgctgcgg ataatgggac gatgtcgttt 1021 tyttagtatt tacttiggog tatatatytg gatcgaataa tasacagaaa cytacyttyt 1081 cyttytgagt gtgagtacty tattattaat gyttotattt gittitactt goaagittic 1141 tigtittigaa titgittitt toatattigi tatotgatto gitogattatt gitättätti 1201 aatttgtaat aagattatgt tacctttgag tggttgttta tcatactttt tttctatggt 25 1261 aagaggtttt ggaaaagtat cgagaatgat atataaagta attttgatat cgacgcaaga 1321 tgataactac tagactagct gagtataaga atattgatgt atatatttgc ggacaattt 1381 gaatttatta taccattatt taatcacgac catataaaa taattcttgt ttgcgttata 1441 atttgtgtta atacgataga gtagacaaat ga (SEQ ID NO: 7)

MATSKALTASLLISLIVIOLVOA

DVENSQKKNGYAKKID

30

CGSACVARCRLSRRPRLCHRACGTCCYRCNCVPPGTYGNYDKCQCYASLTTHGGRRKCP* (SEQ ID NO: 8)

D. At2g14900

1 ataactaaca atggttgagt ggagatgigc ttttagtcaa gtggttaaat atatttgact 5 61 togtttttt cattggagtt tgactotact aagttgtgtt tootogcgta gtaagaattg 121 gttatggatt agaccgtatc gatctamaga tgtcaaagaa amaamaatgt ggttgtgtam 181 agtawatato tagattotog cogattaman tatottitoa ticacatcat tattottatt 241 ttttcatgaa ttctaaatgt aaagttctta taatcttatg ttacttttta caaattgtaa 301 ggattactot gaaatttggt atcgaattot aagacaaata caaaataaca atgactgaac 10 361 aagttgataa aacataatgg aaggaataat actgcagttc tattaaatac taaagaagtt 421 ggtagattgg cctataaaag gagastaaag agaccacaag aaggtctatt attoggggac 481 taaagaaagc caaagaaaac ATGAAAATAA TAGTCTCCAT CTTAGTGTTA GCCTCTCTTC 541 TTCTAATCAG TTCATCTCTT GCTTCGGCTA CTATATCAGG ttggttctaa tctcttcaag 601 aatotictic tototattit tittitotto ataaagitag tiatgitatg attggtitag 15 661 gtcacaattg tttctttatg ctttcgtttc cataagaaaa atattacaaa tattaactag 721 aacaacataa catgcaaacg agtaatacaa aattcattat tatgatcaaa acaatcatga 781 attagttgga cttatttgtt aaattccgaa aatctcacta aaataaagtg aacttcatct 841 acatggottt agacqcaaaa totttaaqqq tatotacaca agtttqqaat qaataattte 901 ttgcgatggt agtgtagaag gatctagaag atccacaaga tcattagtgt atcttctaga 20 961 tccttttaca ttgagaagtg aggagatatt tgttgtatta gaaagaatta tagtgaagta 1021 aatttittaa ctatgtacga toattiatat acgatacttt tattaaggat cttgtggatc 1081 ttctagargc TTTTGGTAGT GGCGCGGTAG CTCCGGCACC GCAGAGCAAA GATGGACCGG 1141 CGTTAGAGAA ATGGTGTGGA CAGAAATGTG AAGGGAGATG CAAAGAAGCG GGGATGAAAG 1201 ATCGGTGTTT GAAGTATTGT GGGATATGTT GCAAAGACTG TCAGTGTGTT CCTTCAGGCA 25 1261 CTTATGGGAA TAAGCATGAA TGTGCTTGCT ATCGTGACAA GCTCAGTAGC AAAGGCACTC 1321 CTAAATGTCC TTGAttctat ttctttccaa ccaasaattt saataaatga ataagagaga 1301 tocagtamac tmatatamam ctatamatgg atottttgtt tatgattttt tttttttcat 1441 tectattett acquattegt ceteggecett etgaagtaag tetetaaata etgaaaagtg 1501 ctaaaattat gtggaaatcg ataatgttaa tgaatgatat aatatataag tootcagttt 30 1561 tigtaagaaa citgaatata aataatatti catcaaacat aataaataaa talatigtat 1621 aattagattg gctcaaccga tataaacaat tgaatcgaat tttttcttct aaatatttaa 1681 tcatccaaat ttgtattgta ccaatgaatg agatggttat gaggactaga agatagagag 1741 gagaagaacg tgtttqqtaa aataattatq atggagttqa qacaactttt aaqagatttt 1801 aaaaagactg actaacgtgt taggttcatc acgt (SEQ ID NO: 9) 35

MKIIVSILVLASLLLISSSLASATIS

DAFGSGAVAPAFQSKDGPALEKW

40

CGOKCEGRCKEAGMKDRCLKYCGICCKDCOCVPSGTYGNKHECACYRDKLSSKGTPKCP* (SEQ ID NO: 10)

E. At2g18420

1 gccaatgggt aactgaggaa gaaggataag accaaaaaa aaactaaaat ggacagattg 61 aattagtaaa aagataaatt ctaaaaaccg aaacaaatct taagttggtg tatatacatc 5 121 tgcattgacc aacaaaagaa agtagactga aatttatttg aaaatgatct tgtaaaggca 181 tattatatat ttaatttagg aaatgaatgt taaateettt aaattgtttt gattteacaa 241 aaggataaag aaatattggt tacatacatc ttaatgtgtt gaccaaaaca aataaaatgt 301 gataaganac aataaaacca ttttgaccaa agttottata gttttaatat totttaattg 10 361 tcatttgtta gtgactaata atattacatt aaacctaatg tataaataga agccccatct 421 totacqcott tataattago aacaaccaaa aacattcatt tgtcattttg totoctottt 481 tgttttctct gatcactagt ATGGCTGTAT TCAGAGTCTT GCTTGCTTCT CTTCTCATAT 541 CTCTTCTTGT CCTCGACTTC GTCCATGCCG ATATGGTGgt acaattttaa caaccaaata 601 tattttctta titgatttta tittttcaca actittgtct acgitctaat ggaattittt 15 661 tcasastatt catgosgaCG TCGAATGACG CCCCTAAAAT CGGtastatc totatostat 721 aaacacgtac gttgaatttc tatatacgtg tgtttaattg aagttttggt tggaaattgt 781 atgtatttgt agattgcaac agcaggtgcc aagagCGGTG CAGTCTTTCG AGTAGGCCAA 841 ATCTTTGTCA CAGAGCGTGC GGGACTTGCT GCGCTAGGTG CAACTGCGTG GCACCGGGCA 901 CATCCGGAAA CTACGACAAA TGTCCGTGCT ATGGTAGCCT AACCACCCAC GGACGACGCA 20 1021 ttatgagagt aattgtggtt attttcttgg gaattattaa aaagcaaaag aaagagaatg 1081 ttatacgtca tgtgcaactc ttcgatcttt gttttagtgt ttatccaatt tgtactigtt 1081 tatacogtca tgrgcaactc ttcgatcrtt gttrugggt tratcosatt igracusgit 1141 ggttrggtt ctggttaaca ttaggtctga aaaggtattg tttttcatta kacaattcac 1201 taaataggca tcgtacttgc atataaaata aaggatpaag agagagatsa aagggttte 1261 tttttttact catggaagtt aggcaatggg ttlaaatatg gtaacaacag aattggaggg 25 1321 gacttaatga actatgacgt aaaactgaga gegattgaat atgtaacgtt accaacaata 1381 ccaataaaat tatgaaagat agtatatgaa attacgttta attaatgttt ccgggttgaa 1441 tgtattatat atagaagtsa cagtacgatt tttattacat ttttgtacaa gattcctaga 1501 aaggtataac ctctataaag ttaataatag tcttgagtct tgactcttcg aggcaaataa 30 1561 attcaccgca twattaatcg ttcaactatt attctatatt ctatataaca tgagcttcaa 1621 caaaagaagc atcaatcata tottcaacag tatactgcag tgtaatgtaa catattcaag 1681 atcaaaccgg acaasaaagc aagataccgt cgaascaatc asaccccatg tatcataaac 1861 atcasaccegy acadeaeage wayneesegs gettgeacaa to (SEQ ID NO: 11)

35 MAVFRVLLASLLISLLVLDFVHA

DMVTSNDAPKID

CNSRCQERCSLSSRPNLCHRACGTCCARCNCVAPGTSGNYDKCPCYGSLTTHGGRRKCP* (SEQ ID NO: 12)

F. At2g30810

_	1	cttttatttg	tttgtgaaaa	aaaacaatag	cttttatttg	tcctaggaat	tatttaatag
5	61	attaaataac	agctattttt	ctcttatttc	ttagtgatta	aaatatttaa	aatacagacc
	121	aaaattaatt	gtttatgtta	atatatttac	tecttaatee	tttatattaa	aattgtataa
	181	tgcatgtagt	taataaattg	ttttccaaaa	ttcattcata	attttattcc	taaattattt
	241	tggtcaagaa	aacacatctt	tgaataatta	aatgcttcct	tgtatttgat	aatttcttga
	301	tattttaaaa	taccttctat	actatgccaa	tgttattggt	tataaatagg	tttaacattg
10	361	atcctgaaat	atatcataag	aaaatcaaaa	gtgaaataag	agatcaaaAT	GATGAAGCTC
	421	ATAGTTGTCT	TTGTTATATC	CASTITICITIC	TTTGCTACTC	AATTTTCTAA	Tgtaaaaatt
	481	attattattt	tettcatatt	atgatttatq	aattcagaga	aataaagttt	ttttttttat
	541	gtgtgtatgt	acagGGTGAT	GAATTAGAGA	GTCAAGCTCA	AGCACCTGCA	ATCCATAAGg
	601	tatatttaaa	ttataasata	tcaaatactg	aataataaat	aataaatata	ttacaacaag
15	661	aatatcaatg	ttatttttca	aactacataa	ttttaaaata	ttttattgat	aacacaaatg
	721	tatattatta	tcgtctccat	tgatttgcat	tctaaatttg	tttttgttat	ccaaccaatt
	781	tcagAATGGA	GGAGAAGGCT	CACTTARACC	AGAAGgtaaa	ttgtttaaaa	gatattattt
	841	ttatttatat	agtaaatgat	tgatcaaatc	acaacttaaa	taatttaatt	gttgatttat
00	901	atttttctga	agAATGTCCA	AAGGCATGTG	AATATCGATG	TTCGGCGACA	TCTCACAGGA
20	961	AACCATGTTT	GTTTTTTTGC	AACAAATGTT	GTAACAAATG	TTTGTGTGTA	CCATCGGGAA
	1021	CATATGGACA	CAAAGAAGAA	TGTCCTTGCT	ACAATAATTG	GACGACCAAA	GAAGGTGGAC
	1081	CAAAATGTCC	ATGAaaacaa	aasattgtaa	aagcaaaata	aaatctatcg	ttgttatctc
	1141	tcaataaaat	ctatgtttgt	aatccttgtt	tttcaatata	gaatataata	tggagttttc
25	1201	ataatttctt	ctattacaaa	attaaagtta	atgcacaaat	aaattgaagg	gacttggacc
20	1261	ttttcgtgta	agttctttct	ttaaatcacg	aacaatttag	atttatattt	tcactcttac
	1321	aaacacaaaa	catggatgct	ctttaactct	catccaaaca	aaatgcattt	ctctctttct
	1381	ttttctaaac	atttcacaac	aatateccat	attatatcta	agatatatga	tctttttaaa
	1441	ttgaatttat	ttaggccatg	ttttaaaatc	gtgtttggtt	agattgaccc	atgaaatgtt
30	1501	gacatattt	aacattccta	aatatgacta	aaaatgatta	aagatattta	ataatatatt
00	1561	tgctctatta	aaaatgatta	aataaataat	aata (SEQ	ID NO: 13)	

MMKLIVVFVISSLLFATQFSNG

DELESQAQAPAIHKNGGEGSLKPEE

CPKACEYRC8ATSHRKPCLFFCNKCCNKCLCVPSGTYGHKEECPCYNNWTTKEGGPKCP* (SEQ ID NO: 14)

40

35

G. At2g39540

1 taatgotata otittaatot ataatatata tragatgiga otitaaggaat ticaatagtt 61 atacataata ataasaatga ataittigita gigitacaaa otigigigica taatcatoat ol diacatasta stassaatga atattutta gigtiacaaa cigigigia 121 teateaggat ticaaaaata teteaaaatt gitgiaagit eatgtaatte gaaatgaatg 181 teaerataa gaastaaatt tacaatttaa aaaatgette aatactggt acaaaaaaaa 5 241 cttcaatac tagtartata ctacttactt agtcaaaaaa gtrtatgaat atggtttttt 301 ctgtatgtta atatttttaa ctgaaaatag taccgacata acaagtaaag atatctttat 361 traangtanc aaacattaat thomoticaa strotcacta traaggatto chotottogt 421 agccacatti caccateact actitigith ogcatacti taaattigi abacqtagg 481 aactottog agamaacmag ATCANGCTOG TGGTTGTMCA ATTCTCTATA ATCTCTCTTC 10 541 TCCTCACATC TTCATTTTCT GTACTTTCAA GTGCTGATTC GTgtaagtgt tracttaatc 601 tagttaataa ttgtaggtca tgcatgtatc attttgaaac aagttttctg aaattctaag 661 atttacata tatatgtgat asatgsatta gcagCATGCG GTGGANAGTG CAATGTGACA 721 TGCTCAAAGG CAGGACAACA TGAAGAATGC CTCAAGTACT GCAATATATG TTGCCAGAAG 15 781 TGTAATTGTG TTCCTTCGGG AACTTTTGGA CACAAAGATG AATGTCCTTG CTACCGTGAT 841 ATGAAAAACT CCAAAGGTGG ATCCAAGTGT CCTTGAacgt tetttgaaga teetcatcac 901 atecetata chichagia citatagigi ggaatatta ateacatta atittigaa 961 tatataaat aaastcaati coccaatii tigaaatett caatigigata citatatiga 1021 tatacagaat aaaastcatti caccaatii tigaaatett caatigigata citaattat 1021 tatacagaa 20 1141 ttgcgccgtt ttgtgattat acasaqtaac atcgtgctgt ttttgacttt tgaaaaccac 1201 agatocaaaa actgtttact tteetetaag agaaagcaaa geegagtgag teeaagegag 1201 adjatetasaaa aetyetesaet caetaecgga gaacgacget atgteagaga eegeegtyte 1321 aategatteg gaecgateta agteggagga agaagaegaa gaagagtatt etecae (SEQ ID NO: 15) 25

MKLVVVOFFIISLLLTSSFSVLSSA

30 DSS

35

CGGKCNVRCSKAGQHEECLKYCNICCQKCNCVPSGTFGHKDECPCYRDMKNSKGGSKCP* (SEQ ID NO: 16)

H. At3g02885 (GASA5)

5 1 cgctttctat tacacttttt tttcttttta gtcgcacttc acaattagct taattaattt 61 cctaaactcg cttattttcc cctttctata tacagatatt atcattagtg acattttcat 121 titiccasaca gagogittag acactagica actacacaat ataatitico sattitoaci 181 gagagamatg tittittit tittitccas ggcaagatit tagictitig gitcictata 241 cytogqtaat tagtgattag taatttacac tgttgagtct ttgacattgt ctaagagaca 10 301 aaaacgacaa gtgtggtacg taattagaaa ttaaaatgac ctacttcccc agaatcacgg 361 catgaacatt ggcaatacca aatttettga ataccattga aggaaateca cactaatcat 421 tttctctata aatatcttta atccgtttta ttgtttctta agaatcattc attggcaatc 481 aagatttttt aaccaaassa ATGGCGAATT GTATCAGAAG AAATGCTCTT TTCTTCTTGA 541 CTCTTCTCTT TTTATTGTCA GTCTCCAACC TCGTTCAGgt aaaccactca aaacagattc 15 601 agtttattaa agtctgatat tgaagtttta tatattacag gctgctcgtg gaggtaaaaa 661 tgaccaaagg ctatacattc cttaaaaatt taatggctat tagttttctg atattgaagt 721 tttatatata tatgacagGC TGCTCGTGGT GGTGGCAAAC TCAAACCCCA ACgtacqgac 781 tcaaaacttt tgttgtttca tatgatcata ttaatttatt aatcactaat tattgataat 841 gttgataaat aaactttaaa gtaacaataa tggtgtttat tttgtgaaat gtcagttttc 901 tagtatactg tatgctgtga attataagca tgaacataaa gatctcaatg atttgttttt 20 961 tgtttgtttg ttgtgatatg cttttttgat ggaaacttca attgtagAGT GCAACTCAAA 1021 GFGTAGCTTC CGTTGTTCAG CAACATCACA CAAGAAGCCA TGCATGTTCT TTTGCCTCAA 1081 GTGTTGCAAA AAATGTCTTT GTGTTCCTCC TGGCACTTTC GGCAACAAAC AAACTTGTCC 1141 ATGTTACAAC AACTGGAAGA CTAAAGAAGG CCGTCCAAAA TGTCCTTAAB acttctttt 1001 againtatt galaatatt attetagttt ggattatoaa acacttacta ototgittia 1261 atcigittet acaagitige gattigicte tacactitii tigigictii tigotettaa 1281 igityigiti gitalaagig laagocogoc oaatgigios igyocogaact tatlatiggit 25 1381 acatatttat gaaatgggct tcattatcaa ttgatttgag cctacaaaaa tgtagccata 1441 aagoccatta agttgtaatt gttaatattt cagtcatasa tatgattttc tatatctatg 30 1501 atttatetet agtgitgatg atgtitgtat giggaagtea tgtietatit getteeaegg 1561 ittaaamace atemeetige taaggicaam itelamint actgigaamm acattatita 1621 cgtgcgtaat tatatgaatt tatgaatagg tittaattoc attitttoct aatagtgtit 1681 tatgtcasa (SEQ ID NO: 17) 35

MANCIRRNALFFLTLLFLLSVSNLVCAA

40 RGGCKLKPOO

45

CNSKCSFRCSATSHKKPCMFFCLKCCKKCLCVPPGTFGNKQTCPCYNNWKTKEGRPKCP* (SEQ ID NO: 18)

I. At4g09600 (GASA3)

_	1	taggctggca	atttaactct	gagacgtctt	tcttgtatag	agaataaaac	atacgcgtgt	
5	61	aaaagaaaac	gcgtgaatcg	aatgatgagt	gttaacgttc	gatcgagatg	ccaccaaatc	
				ggaggacata				
	181	gacatgtgga	ctctactttg	ggtggcatgt	tcatatcttt	ccacatcacc	atgtaaacgt	
	241	'gaaaacaccc	accacactca	cttacatete	aaacacatgt	cttcattatc	gtacgtaget	
	301	ccaaaaaaaa	aaatgaaaac	taggtttagt	gattctattt	cgcaatqtat	aatatacaac	
10	361	ttgtaaaaat	aaaatatttg	aataagcatt	ataaataaac	ccaaagaggt	gttagattta	
	421	tatacttaat	tgtagctact	aaatagagaa	tcagagagaa	tagttttata	tcttgcacga	
	481	aactgcatgc	tttttgagac	ATGGCAATCT	TCCGAAGTAC	ACTAGTTTTA	CTGCTGATCC	
	541	TCTTCTGCCT	CACCACTTTT	GAGgttcata	acttttqtct	ttacttctcc	atgaatcatt	
	601	tgcttcgtct	tatccttaat	tcatatqtqt	ttgatcaatg	ataataattc	atcattctct	
15	661	tcaqCTTCAT	GTTCATGCTG	CTGAAGATTC	ACAAGTCGGT	GAAGGCGTAG	TGAAAATTGq	
				gtaaagtgtt				
	781	gttaaaaact	agtcatatgt	gtataaatat	atcatgtgaa	GATTGCGGTG	GGAGATGCAA	
	841	AGGTAGATGC	AGCAAATCGT	CGAGGCCANA	TCTGTGTTTG	AGAGCATGCA	ACAGCTGTTG	
							GTCCTTGCTA	
20				GTGGCCGTCT				
				gagcacacac				
	1081	tatgaggagg	tataaacaaa	ccagaagtta	atgottcato	ttgaactagt	ataanttota	
	1141	tgaactgtgc	tecttteaa	caaccacttt	toctotagot	ttagcaaccc	tatttaataa	
	1201	attagagatt	acassassas	aaatgaaaaa	totttaaaaa	acotogattt	ttaaatttgg	
25				tttggttgat				
				gttaaatttt				
	1381	ctotaaatat	ctaacttcac	ttcaatctca	caaacacaco	aatcaacttc	aggactaaga	
	1441	atcoaattoa	ccagaactga	aagaaagtaa	aagaaaagct.	gantacagag	aatttaacga	
					,	,		(SEQ ID NO: 19)

MAIFRSTLVLLLILFCLTTF

ELHVHAAEDSQVGEGVVKID

35 CGGRCKGRCSKSSRPNLCLRACNSCCYRCNCVPPGTAGNHHLCPCYASITTRGGRLKCP* (SEQ ID NO: 20)

J. At4g09610 (GASA2)

_	1	ttaacagttt	aacaccataa	tgttaaactc	ggtttagcat	tttggtgtaa	ttctacctct
5	61	ttaaccatac	atactaaaga	cgcagagaag	ttcatatggt	agttaatcgt	aaatagotaa
	121	acttttaatt	ggggttaaca	tattatttaa	cacttaacat	ttmactattg	atctctcatt
	181	ttttttttat	taaccaaaat	aaattcattt	tagaaccaaa	cgtttcaaaa	actcgtaatg
	241	ttttctcatt	asstcttatc	tatageteac	acaaagaaaa	actacqqaca	tgcatgcacc
	301	caattatata	catggattat	tatttttagt	gttataatat	qatacaaaat	aaaaacatt
10	361	tggatageeg	ataggcgata	gccactataa	atataccaaa	qaqqttqqat	tatacatata
	421	googtaatac	caaagagagt	atcagataga	aatagttota	atattttgta	caactcacag
	481	aaattgcatg	agtttcgaac	ATGGCAGTCT	TCCGAAGTAC	ACTGGTTCTG	TTACTAATCA
	541	TCGTCTGTCT	CACCACTTAT	GAGGtttata	atatttttgg	tetttatagt	teccenagaa
	601	cacctagcaa	tattatactc	aattcatqtt	tatatgataa	tgactgatca	ttctcttcag
15	661	CTTCACGTCC	ACGCTGCTGA	TGGTGCAAAG	GTCGGTGAAG	GCGTAGTGAA	AATCGqtatq
	721	taaccctaac	ttatatataa	cacgttggta	tataacttaa	tatttctgat	ggqtqcactc
	781	tcttcccaac	ttatatatat	ctttgttatg	gagaatgtct	caagetttta	atgagatgtt
	841	atatctcgga	gaaggaaact	atgaactaaa	agetttggat	tootttgcaa	caaatataaa
	901	cttttgatgg	gtttaaacgg	attaaattag	ttacatgtgt	ttgatgaatg	tatgtatgat
20		tgtagATTGT					
	1021	CTTGAGAGCG	TGCAACAGCT	GTTGTTCCCG	CTGCAACTGT	GTGCCACCTG	GTACTTCTGG
	1081	AAACACCCAC	CTTTGTCCTT	GCTACGCCTC	CATTACCACT	CACGGTGGCC	GCCTCAAGTG
	1141	CCCTTAAaat	ttcttctgtg	tctgtttctg	tttctacttc	tatttcqaat	atatotacat
	1201	gtgtgtgtac	gtgtgtatgt	atacaagtac	tgctatgttt	tggaggacaa	aagtatatgt
25	1261	atgagaaget	ataaactaat	tagaagttga	tggttatgcg	tattatcaaa	ccgtgttact
		tetgaacaac					
		gattggagac					
		tttttgaact					
	1501	tggttttgta	atggatatga	ttcggatcta	ttctggaaat	ggtctcaaaa	agtagagttg
30		agatotoaat			ttgatttatc	aaagcctttt	attttgaaaa
	1621	cgttaaatcc	tcactaggat	ctctctt /C	EQ ID NO:	21\	
				10	LQ 10 140.		

35 MAVERSTLVLLIIVCLTTY

ELHVHAADGAKVGEGVVKID

CGGRCKDRCSKSSRTKLCLRACNSCCSRCNCVPPGTSGNTHLCPCYASITTHGGRLKCP** (SEQ ID NO: 22)

K. At5g15230 (GASA4)

1 aaatatteac ootaaaatga atotaaaaat gtacaaaato acaggaaaat aaaactaago 61 aqaaatgtoo taagaaaact aaagttttta aaaaataato ttoaaagaga tactocaact 5 121 ggtgttataa gcasaacttg attiatcaaa aacaggttca tagtattita tattiagtac 181 tataaggttt ccttaaacca tgtgcaaaac catctaccgc agtctaatta ccaatagcaa 241 gtaataaaat gggactaaca ttggaggcat acgtggaata atataattqq aqqaatacag 301 taataatgat atgtgttgcc acagggaata attgatacga gcaaatgtgt gtatatatag 361 citalataga acatcattgg gioctraacc anamacung yonaciggg gialatatag 421 atactcang agactaans tagittgag gasttanga gastgittgg tictitic 481 acatcatac canactgan ArGGCTAACT CATATGAGC TACTCTCT TYGACCTCA 10. 541 TTGTCCTCTT CATGCTTCAA ACCATGgtaa cacctctatt atttttttct tetttcaatg 5-11 TENCOCKTY OMGGYTCAN ANGANGGING GOCCOLARL attRettact tettacquae gatatquige Col tttgaagaat tetgaagata tatatitgat (pttttoct) attgaagat gatatquige Col angatquige Colorate (Sanatquige) colorate (San 15 901 taccttttt ggttaatttt cagAAACGTT ATGGACCAGG AAGCCTGAAA CGTACCCgta 20 961 agttttttct tcacagctat tcttaaacaa tttttttta atctcataat cyacgaaaaa 1021 tamacantto amgagatott ttattgtgtt ataatamama ammatamgca tttcagttgc 1081 agaaaataag tigaaagtga agtyttaagt ggactgittig gicagatccg tagactcaaa 1141 atatattaga tattgacgaa attgcccctt aatatggtca tacagtcaaa gcaaccact 1201 atcttgagac ccacaaaaca gtaaaaaaaa aagctaatga atttccacta gattctgttg 1261 tuttattag taataaaaa tuttagatg titaacatut gatatigtit giattigaa 1321 caaccagAAT GCCCATCGGA ATGTGATAGG AGGTGTAAAA AGACACAGTA CCACAAGGGT 25 1381 TGCATTACGT TCTGCAACAA ATGCTGCAGG AAGTGTCTCT GTGTGCCTCC GGGTTACTAT 1441 GGGAACAAC AAGTTTGCTC CTGCTACAAC AACTGGAAAA CTCAAGAGGG TGGACCAAAA 1501 TGCCCTTGAs assatctccc ttcgttccct ttttatasta assattttcs actatascta 30 1561 aattteettt gateaatgtt ttatetactt tatteetaat gttgtaatgt tatgteacte 1621 ottttoggat titgttotaa atootaaaaa aaatgagagt ggoodtatga atgatattit 1681 tostgestac tigigittot asagstatti toccattost coaccassas assagstatt 1741 ttccatttcg aasatagtaa tactataaag ggtaaggcaa accasataat acaatttaaa 1801 aaattootgo gasagaagta tooatatgta gaaaagagtg acattgggto totoggooca 35 1861 gtactaassa goccattatt gatttttccs agctttttac assatcacgt gttctaacgc 1921 gattgctttt tgccgcaatc ttcttttata caagacttgg gctttgggca gttggaaata 1981 aataacgaca acgatatttt acaatcggt (SEQ ID NO: 23) MAKSYGAIFLLTLIVLFMLQTMV 40

Massgsnvkwsqkrygpgslkrtq

CPSECDRRCKKTQYHKACITFCNKCCRKCLCVPPGYYGNKQVCSCYNNWKTQEGGPKCP** (SEQ ID NO: 24)

45

L. At5g14920

1 ttgctcactg gtgcaataat cgaagtgaag agcctcttta tatgaaatat ataagcgaca 5 61 cagcottatg ggcaaatcga atgctattta tttatttgat aagaagatta ataatttcaa 121 tttgtcatcc actagtctct tggggtactc aaaacatatc accaaaaagt ccatagagtt 181 atttgttctt atttactgat adagtattc aagttgatgt acquataaag tggcaatttc 241 atgtattatc atataatcc atttttggga atctgatatt ttgtttatcc tcgagctctg 301 agagatatat titygtgcag tgaaggitca aagciggcat gcatgatgca tataataaci 10 361 octotogaco tastacttac tacqcattta sattastatt tatqqatast atqqttasta 421 aataaggaac ttctatttat atcacaaaag gtcactggtc ttcttcgtgt gacttcacca 481 ctttctcatc tcccacaaaa ATGGCTCTCT CACTTCTTTC AGTCTTTATC TTTTTCCATG 541 TCTTTACCAA Tgtaagttat tcttactttt cataacaaaa ggtgttatta tgttaaagac 601 tacataatag tatacaatta tgtgcattac gttttcgcgt attgtaacta actatgtatt 661 ttrattaatc accaacagg TTGTTTTTGC TGCTTCAAAT GAGGAATCCA ACGCCTTAgt 15 721 acgttttcta atttccagtt taattatttc tatocotctt taactatata ctcaggcatt 721 titaligati attigtatig angitasati tiggitasat titigitatia titigitatia 841 TICTITACCA ACSCCAACAC TICCATGGC ATTICGGCT ACCAACAGC GCTGGCCAGC 91 TICTAACG CCGGGGGGG CGTACAGCC ACCAGGGTG CCAACTACT CTATTANACC 20 961 ACCCACCACA AMACCTCCGG TCAMACCTCC AMCTATTCCG GTTACACCAG TAMAACCTCC 1021 GGTTTCAACT CCTCCGATCA AACTACCGCC GGTACAACCA CCTACGTACA AACCCCCAAC 1081 GCCAACAGTT ARACCACCGT CCGTCCAACC ACCTACGTAC ARACCCCCAA CTCCAACGGT 1141 TARACCACCC ACTACATCAC CGGTTARACC ACCCACTACG CCACCAGTTC AATCACCGCC 1201 GGTCCRACCA CCTACGTACA AACCCCCAAC GTCACCGGTT AAACCACCCA CCACAACTCC 25 1261 ACCGGTTANA CCCCCCACCA CGACGCCACC GGTCCAACCA CCTACGTACA ATCCCCCAAC 1321 TACACCGGTT AAACCACCTA CAGCGCCGCC TGTCAAACCT CCAACACCAC CTCCCGTAAG 1381 AACTCGGATA Gqtaataata attttctttc aaaaqtqtqa tqattatcgg tcgttgatta 1441 gateggatgt ataattggac taaattttgg acggtttagA TTGCGTGCCT TTATGTGGGA 1501 CGAGGTGTGG GCAACACTCG AGGAAGAACG TATGTATGAG AGCGTGCGTC ACGTGCTGCT 30 1561 ACCGCTGCAA GTGTGTTCCC CCAGGCACCT ACGGTAATAA GGAGAAGTGT GGATCTTGTT 1621 ACGCCAACAT GAAGACACGT GGTGGAAAAT CCAAATGTCC TTGAaccttt atatoacgat 1681 ggttgttaaa cgaaataatt taaatcaatg gagtttttat aagtttgtaa tgcgtttgtt 1741 tttgttatag taatattgag ttggatcttt gtttacggga cgtagaatac taaataatga 1801 aaaaaacctt ctcggtgaat taagggtttt atgaatttgt tttgtattga ataatatagg 35 1861 gatggatama gttttattat totamonggt tactttatta ggcatttott oggotomtgt 1921 aactottgta togotgaaac tatgtaatag atagaayaac otaaaaaaag aaagaaaaca 1991 agaaatgcac atagcgaagc tcaaaagatg agtgttctgc tagcggtaat gttgttattc 2041 agttgggtca aatgctctaa ttgcaaatct tatttgggcc ttatatagac tcttatgtgc 2101 atatggtcca gcctatttgg gccgatgtgt ttgaagatca tttgggaaag tcttgcgcaa 40 2161 ggag (SEQ ID NO: 25)

40

MALSILSVFII'FHVFTNVVFAAS

45 NESSNAVSLEPTELSSESA
TROBENLKOPTOSKYDPILA
TTPIKPPTKOPVKPPTIPVT
VKORVSTPIKLEPVQDFTY
KPPTTVKPPSVQDFTXKPT
TVKPTTSPVKPPTTPVQS
PPVQPTTKPTSPVKPPTTPVQS
PPVQPTTKPTSPVKPPTTT
PVKPPTAPPVKPPTPPVAT
RID
55

CVPLCGTRCGQHSRKNVCMRACVTCCYRCKCVPPGTYGNKEKCGSCYANMKTRGGKSKCP* (SEQID NO: 26)

M. At5g59845

	1	gacttgagta	tgaatccaat	aacccaaaat	ttatgcagat	tttagaatac	ttcttataaa
5	61	tcttaaatga	ataacacaaa	actttaacat	acttttaaca	aatcttgatt	gaataacaac
	121	agattc taca	tgacatttta	aatcactaaa	actettttqa	aatcataaac	caataacaac
	181	cccttagttt	tttactattt	gaattctgac	gtactttttt	attagttgaa	tttctataaa
	241	t gagaaaaca	ttaattattt	cttaatcttt	quacttaage	cccacaaaaa	tcttataaat
	301	tgggacagat	ggactagata	acaagcgttt	cacctactcc	aaaatttccc	tataagtaac
10	361	tetttttgta	acctcctttt	cttcccaaac	catcactcct	tttgcattgt	gtgaaacct t
	421	cgagttttct	etteatette	tcaaagtaac	aaactttete	caaacagatt	attattaaaa
	481	caatctcatc	aagaactacq	ATGAAATTCC	CGGCTGTAAA	AGTTCTTATT	ATCTCTCTTC
	541	TCATCACATC	TTCTTTGTTC	ATACTCTCAA	CCGCGGATTC	GTgtaagtat	acacaateca
	601	ttttcttatt	ttagatactt	ttctcattag	agatttaget	ttcttaataa	aattotatto
15	661	tgatgatgga	ttaattagCA	CCATGCGGAG	GAAAATGCAA	CGTGAGATGT	TCAAAGGCAG
	721	GAAGACAAGA	TAGGTGTCTC	AAGTATTGTA	ATATATGTTG	CGAGAAGTGT	AACTATTGTG
	781	TTCCTTCAGG	CACTTATGGA	AACAAAGATG	AATGCCCTTG	TTACCGCGAT	ATGRAGARCE
	841	CCAAAGGCAC	GTCCAAATGT	CCTTGAt cat	gttcttaaga	ttatocttat	agacacaata
	901	tcttgaaatg	ttaagattot	gcttgatgcc	taaaataato	agettgagat	act totates
20	961	atgaatatgt	Canagatttt	gacaataaaa	tgatttgatg	tattaaaata	ttetteatas
	1021	agttatatat	gtataaatga	agtatgaaat	atacattota	tottocttta	catazassa
	1081	ataaatctac	aacaatccaa	totatoaaaa	ttttactaac	ttaactcatc	agazagatta
	1141	attatggttt	agaatettot	nnacanat na	ttacttttct	aggagagatt	ayaaacytta aattattiat
	1201	tgtcaatgag	gataaagtaa	gaagecattt	ctcaacacat	agagasacc	gactgetege generates
25	1261	caaggeteaa	gcattoggat	trassentet	contacutus	gattagata	2022222000
	1321	gtgttttttg	ttgtagaaaa	cagaaattga	aattactato		
	1021	y-y		ougunactya	auceuc tyte	(SE	Q ID NO: 27)

30 MKFPAVKVLIISLLITSSLFILSTA

DSSP

CGGKCNVRCSKAGRQDRCLKYCNICCEKCNYCVPSGTYGNKDECPCYRDMKNSKGTSKCP* (SEQ ID NO: 28)

N. At3g10170

genomic structure before splicing and processing 5^\prime - towards 3^\prime predicted orf sequences are underlined

5
CROTTICAGAMANGGCACAMACTIAGCATCATTGTTTCCCCATTG
TGGTTACAACTCTCTGTCGCCCATAGCATCATAGCATGTTTCAACA
CCCTATCCCCTCATGTCCAGATTATACATTCTTATCAGCCCTCA
CCCTATCCCCTCATGTCCAGATTATACAACTTATCATACTGCCCTCA

20 CITTAGUATARACTUCATGATATTGTTAATCTACCTATCATAATTTATA
TATGTATTGGACTCTCCATAATCACATCAGTTCTCTGTGATTATGACGT (SEQ ID NO: 29)

Amino acid sequence of the predicted pre-pro-peptide the first line represents the signal sequence

the second (set of) lines represents the the pro-peptide 25 the last line represents the conserved Cysteine motif.

MATKLSIIVFSIVVLHLLLSAHMH

FLINVCAECETKSAIPPLLE

CGPRCGDRCSNTQYKKPCLFFCNKCCNKCLCVPPGTYGNKQVCPCYNNWKTKSGGPKCP* (SEQ ID NO: 30)

They consist of an N-terminal signal peptide, followed by a variable domain (involved in mobility or cell wall attachment)

5 and a C-terminal domain with 12 conserved cystein residues.

The Consensus of this last domain is:

C-C-RC-----G--KCP* (SEQ ID NO: 31)

- (-) = any amino acid;
- (C) = conserved C-residue
- 10 (/) = either one or the other amino acid at this position;
 - * = stopcodon

Some members of this gene family have been described previously, and represent the GASA family in Arabidopsis 15 thaliana (Plant Mol. Biol. 36 (1998). Similar family members containing the same structural motifs are present in rice (like GASR1) and tomato (Plant Journal 2 (1992) 153-159; Mol. Gen. Genet. 243 (1994) Taylor and Scheuring). In Arabidopsis, the GASA gene family represents 14 different 20 membres, similar as the number for the RKS gene family. Our data on the similar phenotypes for RKS4 and GASA3 (figure 6) and the fact that there are similar numbers of ligands and receptors suggest that there is a single GASA ligand molecule interaction with a single RKS molecule. T-DNA knock out 25 phenotypes observed with several of the other GASA peptide ligand genes also show modifications of organ and plant size like the appearance of extreme dwarf plants resembling brassinosteroid insensitive mutants. Co-localization of RKS genes and GASA ligands on the genome (see figure 4) could 30 provide clues of molecular interactions between GASA molecules and RKS molecules (similar as for S locus proteins and S locus receptor kinases). Furthermore, in the chapter discussing the effects of roots in RKS transgenic plants, it was shown that overexpression of RKS genes can result in the formation of lateral roots (figure 26). One of the GASA ligands is involved in the formation and/or outgrowth of lateral roots as discussed in Mol. Gen. Genet. 243, 1994, 148-157.

Intracellularly, this signal is transmitted onto membrane (but not necessarily plasma membrane) associated NDR-NHL proteins. At least some of the functions of the syntaxin-like NDR-NHL proteins would thereby result in the regulation of vesicle transport and /or the positioning of new cell wall formation. Neighboring cells are known to influence and determine the developmental state and the differentiation of cells. In transgenic plants with RKS and / or NDR-NHL expression

10 cassettes the positioning of new cell walls is modified, resulting in abnormal neighboring cells, resulting in abnormal development of groups of cells like flower meristem primordia as observed and shown with RKSO, RKS13 and NHL10.

genes in arabidopsis and in rice: overview of accessions numbers of RKS signal complex Table 2

	Tanta	7 000	ew or accessions	numbers of two	erginar compr	TABLE & OVERVIEW OF ACCESSIONS NUMBERS OF INC. SEGMAL COMPLEX Genes in GrabioOpsis and in thee.	and in pice.
	Gene	Gene code	contig	gene prediction	g	Oryza sativa	approximate position
10				in At database		japonica contig	in bp around:
	RKS0	At1g71830	f14o23	ok		OSJNBa0036B21	52.000
	RK31	At1960800		ok		P0038C05	000.09
	RKS2		mqn23	ok		OJ1212 C08	8000
	RKS3	At5g63710	mbk5	ok		see rks2	
9	RKS4	At2g23950	t29e15	wrong, exon missing	ssing	P0708B04	35.000
	RKS5	At5g45780	mra19	wrong, exon m	exon missing	OJ1077 A12	102.000
	RKS6	At5g10290	wt e 23	ok		see rks2	
	RKS7	At5g16000 ku e 24	ku e 24	ok		P0038C05	000.09
	RKS8	RKS8 At1g34210	£23m19	ok		OJ1134 B10	90.000 & 1000 2
12	diffe	rent genes				ı	
	RKS10	RKS10 At4g33430 en d 25	en d 25	wrong, exon m	exon missing	see rks0	
	RKS11	RKS11 At4g30520 wu d 20	wu d 20		exon missing	see rks4	
	RKS12	KS12 At2g13800	£13j11		exon missing	see rks10	
	RKS13	RKS13 At2g13790				P0633E08	36.000
8	RKS14	RKS14 At3g25560	mw12	wrong, exon m	exon missing	OSJNBD0015G09	36.000
	ELS1	At5g21090	At5g21090 ch e 52 ok p0003H10	ok		P0003H10	53.000
	ELS2	possibly a	allelic variant o.	f ELS1 no genom	ic sequence	identified yet	see els1
	ELS3	At3g43740 by c 21	by c 21	ok		P0468B07	52.000

Homology between as sequences from arabidopsis proteins are compared with the rice databases using: http://mips.gsf.chpio/frame.thml protein sequences based on Oryza safiva japonica contig sequences.

30

25

Arabidopsis thaliana ELS1 cDNA

The start codon encoding the first predicted methionine 5 residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in
capitals. Leader and trailer sequences are in lowercase
10 letters.

gaagaattgggggtgaccttgtaagaacacttcaccactttatcaaatatc

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain
represents a signal sequence. The second domain contains a
leucine zipper motif, containing 4 leucine residues, each
separated by seven other amino acids. The third domain
contains conserved cysteine residues, involved in disulphate
bridge formation. The fourth domain contains a leucine rich

repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The last domain might be involved in attachment to other proteins or structures within the cell wall.

MASRNYRWELFAASL TLTLALIHLVEANSEG

5

20

DALYALRRSLTDP 10 DHVLQSWDPTLVN

PCTWFHVTCNQDNRVTRV

DLGNSNLSGHLA

15 P ELGKLEHLQYLELYKNNIQGTI
PSELGNLKNLISLDLYNNNLTGIV
PTSLGKLKSLVFLRLNDNRLTGPI
PRALTAIPSLKVVDVSSNDLCGTI
PTNGPFAHIPLQNFENNPRLEGPE

LLGLASYDTNCT (SEQ ID NO: 33)

Arabidopsis thaliana ELS2 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 aaaattactcaaattcctattagattactctttcgactccgatagctcac
 ATGCCGTCTCGAACTATCGGTGGGGGCTTCTCGCAGCTTCGTTAATCCTAA
 CCTTAGCTTGATTCACCTGGTCGAAGCAACTATCCGAAGGAGGATGCTCTTTA
 CGCTCTTGGCGGAGTTTAACAGATCCGGACCATGTCCTCCAGAGCTGGGAT
 CCAACTCTTGTTAATCCTTCTACCTGGTTCCATGTCACCTGTACCAGGACA
 15 ACCGCGTCACTCGGGATTTGACGGATTCAAGCTCTCTGGACTCTGG
 GCCTGAGCTTGGGAACTTGAACATTTACAGTATCTGAGGATCTACAAAAAC
 AACATCCAAGGAACTAAACTTTACAGGATACTGAAGAATCTACATGA
 GCTTGGATCTGTACAACAACAATCTTACAGGGATAGTTCCACTTTTTTGG
 AAAATTCAAGTCTCTGGTTTTTTACGGGATAGTACACAACTATCACAGGA
 AAAATTCAAGTCTCTGGTTTTTTACGGCTTAATGACAACCGATTGACCGGG
- 20 CAATCCCTAGAGCACTCACTGCCAATCCCAAGCCTTAAAAGTTGTGGATGTC TAAGCAATGATTTGTGTGGAACAATCCCAAGCAAGGACCTTTTGCTCACAT TCCTTTACAGAACTTTGGGAACAACCCAAGGTTGGAGGGACCGGAATTACTC GGTCTTGCAAGCTACGACACTAACTGCACCTGAagaaaattggcaaaacctga aaatgaagaattgggggggaccttgtaagaacacttcaccactttatcaaat

gaatogaatagtaatatoatotggtotoaattgagaaotttgaggtotgtgt atgaaaattaaagattgtaotgtaatgttoggttgtgggattotgagaagta acatttgtattggtatggtatoaagttgttotgoottgtotgcaaaaaaaa (SEOIDNO:34)

ised in the

30

Predicted amino acid sequence of the Arabidopsis thaliana ELS2 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as 35 described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino acids. The third domain

contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The last domain might be 5 involved in attachment to other proteins or structures within the cell wall.

MASRNYRWELFAASL ILTLALIHLVEANSEG

10

DALYALRRSLTDP DHVLOSWDPTLVN

PCTWFHVTCNODNRVTRV

15

DLGNSNLSGHLA

P ELGKLEHLQYLQLYKNNIQGTI
PSELGNLKNLISLDLYNNNITGIV
PTSLGKLKSLVFLRLNDNRLTGPI
20 PRALTAIPSLKVVDVSSNDLCGTI
PTNGPFARIPLQNFENNPRLEGPE

LLGLASYDTNCT (SEQ ID NO: 35)

Arabidopsis thaliana ELS3 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined.
 - Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 ttetetetegggaaaccATGGTGGGGCAAAACAGTCGGCGGGAGCTTCTAGCAGCTT
 CCCTGATCCTAACTTTAGCTCTAATTCGTCTAAGGGAAGCAAACTCCGAAGGGGACCTC
 TTCAGGGGCTTGGGCGGAGCTTATCAGATCCAAGTTTGTTCAGAGTTGGGATCACC
 CTCTTGTTAATCCTTGTTCTTGTTTCATGTCACTTGTAATCAACACCATCAAGTCACC
 GTCTGGATTTGGGGAACTTGAACTTATCTGGAACTTGGCAAGCTTG
- 15 ARCRITTACARTATCTTGRACTCTACARARACGAGATTCAAGGARCTATACCTTCTGAGC
 TTGGAAATCTGAAGAGTCTAATCAGTTTGGATCTGTACAACAACCATCTCCACCGGGAAAA
 TCCCATCTTCTTTGGGAAAATTGAAGCGGCTTAACGAAAACCGATGACCGGTCCTATTC
 CTAGAGAACTCACAGTTATTCAAGCCTTAAAGTTGTTGATGTCTCAGGGAATGATTTCT
 GTGGAACAATTCCAGTAGAAGGACCTTTTGAACACATTCCTATGCAAAACTTTGAGAACA
- gtggaacattccagtagaagaaccttttgaacacattcctargcaaactttgagaaca
 20 acctgagattggagggaccagaactactaggtcttgcgagctatgacaccaattgcact<u>T</u>
 <u>Aa</u>aaagaagttgaagaa (SEQIDNO:36)
 - Predicted amino acid sequence of the Arabidopsis thaliana ELS3 protein.
- 25 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997). At the predicted extracellular domain the first domain
 - represents a signal sequence. The second domain contains a
- 30 leucine zipper motif, containing 2 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each
- 35 approximately 24 amino acid residues. The last domain might be involved in attachment to other proteins or structures within the cell wall.

MVAQNSRRELLAASL ILTLALIRLTEANSEG

DALHALRRSLSDP

5 DNVVQSWDPTLVN

PCTWFHVTCNQHHQVTRL

DLGNSNLSGHLV

10 P ELGKLEHLQYLELYKNEIQGTI PSELGNLKSLISLDLYNNNLTGKI

P SSLGKLKRLNENRLTGPI

PRELTVISSLKVVDVSGNDLCGTI

PVEGPFEHIPMQNFENNLRLEGPE
15

LLGLASYDTNCT (SEQ ID NO: 37)

Arabidopsis thaliana RKSO cDNA

letters.

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase

10 attttattttatttttactctttgtttgttttaatgctaatgcqtttttaaaaggqtt

 ${\tt atcgaaaaaatgagttgagtttgttgaggttgtctctgtaaagtgttaatggtggtgat}$ tttcqqaaqttaqqqttttctcqqatctqaaqaqatcaaatcaagattcqaaatttacca ttgttgtttgaaATGGAGTCGAGTTATGTGGTGTTTATCTTACTTTCACTGATCTTACTT CCGAATCATTCACTGTGGCTTGCTTCTGCTAATTTGGAAGGTGATGCTTTGCATACTTTG 15 AGGGTTACTCTAGTTGATCCAAACAATGTCTTGCAGAGCTGGGATCCTACGCTAGTGAAT CCTTGCACATGGTTCCATGTCACTTGCAACAACGAGAACAGTGTCATAAGAGTTGATTTG GGGAATGCAGAGTTATCTGGCCATTTAGTTCCAGAGCTTGGTGTGCTCAAGAATTTGCAG TATTTGGAGCTTTACAGTAACAACATAACTGGCCCGATTCCTAGTAATCTTGGAAATCTG ACAAACTTAGTGAGTTTGGATCTTTACTTAAACAGCTTCTCCGGTCCTATTCCGGAATCA 20 TTGGGAAGCTTTCAAAGCTGAGATTTCTCCGGCTTAACAACAACAGTCTCACTGGGTCA ATTCCTATGTCACTGACCAATATTACTACCCTTCAAGTGTTAGATCTATCAAATAACAGA CTCTCTGGTTCAGTTCCTGACAATGGCTCCTTCTCACTCTTCACACCCCATCAGTTTTGCT AATAACTTAGACCTATGTGGACCTGTTACAAGTCACCCATGTCCTGGATCTCCCCCGTTT TCTCCTCCACCACCTTTTATTCAACCTCCCCCAGTTTCCACCCCGAGTGGGTATGGTATA 25 ACTGGAGCAATAGCTGGTGGAGTTGCTGCAGGTGCTTTGCCCTTTGCTCCTCCTGCA ATAGCCTTTGCTTGGTGGCGACGAAGAAGCCCACTAGATATTTTCTTCGATGTCCCTGCC CARGARGATCCAGAAGTTCATCTGGGACAGCTCAAGAGGTTTTCTTTGCGGGAGCTACAA GTGGCGAGTGATGGGTTTAGTAACAAGAACATTTTGGGCAGAGGTGGGTTTGGGAAAGTC 30 ACTCCAGGTGGAGAGCTCCAGTTTCAAACAGAAGTAGAGATGATAAGTATGGCAGTTCAT CCGCTTGATTGGCCAACGCGGAAGAGAATCGCGCTAGGCTCAGCTCGAGGTTTGTCTTAC CTACATGATCACTGCGATCCGAAGATCATTCACCGTGACGTAAAAGCAGCAAACATCCTC 35 TTAGACGAAGAATTCGAAGCGGTTGTTGGAGATTTCGGGTTGGCAAAGCTTATGGACTAT A A GACACTCACGTGACAACAGCAGTCCGTGGCACCATCGGTCACATCGCTCCAGAATAT CTCTCAACCGGAAAATCTTCAGAGAAAACCGACGTTTTCGGATACGGAATCATGCTTCTA GAACTAATCACAGGACAAAGAGCTTTCGATCTCGCTCGCCTAGCTAACGACGACGACGTC ATCTTACTTGACTGGGTGAAAGGATTGTTGAAGGAGAAGAAGCTAGAGATGTTAGTGGAT CCAGATCTTCAAACAACTACGAGGAGAGAGAACTGGAACAAGTGATACAAGTGGCGTTG

5

Predicted amino acid sequence of the Arabidopsis thaliana RKSO protein.

Different domains are spaced and shown from the N-terminus

10 towards the C-terminus. Overall domain structure is similar as
described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino 15 acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline

20 residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat,

probably involved in protein / protein interactions.

30

MESSYVVFILLSLILLPNHSL WLASANLEG

DALHTLRVTLVDP

35 NNVLQSWDPTLVN

PCTWFHVTCNNENSVIRV

DLGNAELSGHLV

40 P ELGVLKNLQYLELYSNNITGPI

PSNLGNLTNLVSLDLYLNSFSGPI PESLGKLSKLRFLRLNNNSLTGSI PMSLTNITTLQVLDLSNNRLSGSV PDNGSFSLFTPISFANNLDLCGPV

5 TSHPCPGSPPFSPPPP FIQPPPVSTPSGYGITG

AIAGGVAAGAAL
10 PFAAPAIAFAWW

RRRKPLDIFFDVPAEEDPE VHLGQLKRFSLRELQVAS

15 DGFSNKNILGRGGFGKVYKGRLAD
GTUAVKRLKEERTFGGELQFQ
TEVEMISMAVHRNLLRLRGFCM
TFTERLLVYFYMANGSVASCLR
ERPPSQPFLDMPTRKRIALGSA
RGLSYLHDHCDPKIIRHDVKAA
NILLDEEFEAVVGDFGLAKIMD
YKDRHVTAVKGTIGHTAFEYL
STGKSSEKTDVFGYGIMLLELI
TGQRAFDLARLANDDDVMLLDW
VKGLKEKKLEHLVDPDLGTMY
EERELEQVIQVALLCTQGSPME
RPKMSSVVRNLE

GDGLAEKWDEWQKVEILREEIDLS

30

PNPNSDWILDSTYNLHAVELSGPR (SEQ ID NO: 39)

Arabidopsis thaliana RKS1 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 ccaaagttgattgctttaagaagggatATGGAAGGTGTGAGATTTGTGGTGTGGAGATTA

GGATTTCTGGTTTTTGTATGGTTCTTTGATATCTCTTCTGCTACACTTTCTCCTACTGGT GTTCTTGAGAATTGGGATGTGAATTCAGTTGATCCTTGTAGCTGGAGAATGGTTTCTTGC ACTGATGGCTATGTCTCTCACTGGATCTTCCTAGCCAAAGCTTGTCTGGTACATTGTCT 15 CCTAGAATCGGAAACCTCACCTATTTACAATCAGTGGTGTTGCAAAACAATGCAATCACT GCTCCAATTCCGCAAACGATTGGGAGGTTGGAGAAGCTTCAGTCACTTGATCTTTCGAAC AATTCATTCACCGGGGAGATACCGGCCTCACTTGGAGAACTCAAGAACTTGAATTACTTG CGGTTAAACAATAACAGTCTTATAGGAACTTGCCCTGAGTCTCTATCCAAGATTGAGGGA CTCACTCTAGTCGACATTTCGTATAACAATCTTAGTGGTTCGCTGCCAAAAGTTTCTGCC AGAACTTTCAAGGTAATTGGTAATGCCTTAATCTGTGGCCCAAAAGCTGTTTCAAACTGT TCTGCTGTTCCCGAGCCTCTCACGCTTCCACAGATGGTCCAGATGAATCAGGAACTCGT ACCAATGGCCATCACGTTGCTCTTGCATTTGCCGCAAGCTTCAGTGCAGCATTTTTTGTT TTCTTTACAAGCGGAATGTTTCTTTGGTGGAGATATCGCCGTAACAAGCAAATATTTTTT GACGTTAATGAACAATATGATCCAGAAGTGAGTTTAGGGCACTTGAAGAGGTATACATTC 25 AAAGAGCTTAGATCTGCCACCAATCATTTCAACTCGAAGAACATTCTCGGAAGAGGCGGA TACGGGATTGTGTACAAAGGACACTTAAACGATGGAACTTTGGTGGCTGTCAAACGTCTC TTGGCTCTTCATCGCAATCTCCTCCGGCTCCGCGGTTTCTGTAGTAGCAACCAGGAGAGA ATTTTAGTCTACCCTTACATGCCAAATGGGAGTGTCGCATCACGCTTAAAAGATAATATC 30 CGTGGAGACCCAGCATTAGACTGGTCGAGAAGAAGAAGATAGCGGTTGGGACAGCGAGA GGACTAGTTTACCTACACGAGCAATGTGACCCGAAGATTATACACCGCGATGTGAAAGCA GCTARCATTCTGTTAGATGAGGACTTCGAAGCAGTTGTTGGTGATTTTGGGTTAGCTAAG CTTCTAGACCATAGAGACTCTCATGTCACAACTGCAGTCCGTGGAACTGTTGGCCACATT GCACCTGAGTACTTATCCACGGGTCAGTCCTCAGAGAAGACTGATGTCTTTGGCTTTGGC 35 ATACTTCTCCTTGAGCTCATTACTGGTCAGAAAGCTCTTGATTTTGGCAGATCCGCACAC CAGAAAGGTGTAATGCTTGACTGGGTGAAGAAGCTGCACCAAGAAGGGAAACTAAAGCAG TTAATAGACAAAGATCTAAATGACAAGTTCGATAGAGTAGAACTCGAAGAAATCGTTCAA GTTGCGCTACTCTGCACTCAATTCAATCCATCTCATCGACCGAAAATGTCAGAAGTTATG AAGATGCTTGAAGGTGACGGTTTGGCTGAGAGATGGGAAGCGACGCAGAACGGTACTGGT 40 GAGCATCAGCCACCGCCATTGCCACCGGGGATGGTGAGTTCTTCGCCGCGTGTGAGGTAT

TACTCGGATTATTCAGGAATCGTCTCTTGTAGTAGAAGCCATTGAGCTCTCGGGTCCTCGGTTCTCTGGATTATATTCAGGAATCGTCTCTGTAGTAGAAGCCATTGAGCTCTCGGGTCCTCGGGTCCTCTGGATTATATTCAGGAATCGTCTCTGTAGTAGAAGCCATTGAGCTCTCTGGGTCCTCTGTAGTAGAAGCCATTGAGCTCTCTGGGTCCT

5 Predicted amino acid sequence of the Arabidopsis thaliana RKS1 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

- 10 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
- bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-
- 20 glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
- 25 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MEGVRFVVWRLGFL VFVWFFDISSATLSPTGVNYEV

TALVAVKNELNDP

35 YKVLENWDVNSVD

PCSWRMVSCTDGYVSSL

DLPSQSLSGT

LSPRIGNLTYLQSVLQNNAITGPI

PETIGRLEKLQSLDLSNNSFTGEI PASLGELKNLNYLRLNNNSLIGTC

5 PESLSKIEGLTLVDISYNNLSGSL

PKVSARTFK VIGNALICGPK

AVSNCSAVPEPLTL PODGPDESGTRING

10

HHVALAFAASFS

AAFFVFFTSGMFLWW

RYRRNKQIFFDVNEQYDPE

15 VSLGHLKRYTFKELRSAT

NHFNSKNILGRGGYGIVYKGHLND

GTLVAVKRLKDCNIAGGEVQFQ

TEVETISLALHRNLLRLRGFCS

20 SNQERILVYPYMPNGSVASRLK

DNIRGEPALDWSRRKKIAVGTA RGLVYLHEOCDPKIIHRDVKAA

NILLDEDFEAVVGDFGLAKLLD

HRDSHVTTAVRGTVGHIAPEYL

25 STGQSSEKTDVFGFGILLLELI

TGQKALDFGRSAHQKGVMLDW

VKKLHQEGKLKQLIDKDLNDKF DRVELEEIVOVALLCTOFNPSH

RPKMSEVMKMLE

30

GDGLAERWEATQNGTGEHQPPPLPPGMVSSS

PRVRYYSDYIQESSLVVEAIELSGPR (SEQ ID NO: 41)

Arabidopsis thaliana RKS2 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

Italics indicate the presence of an alternatively spliced gene product.

10

tcaattttggtagctcttagaaaaATGGCTCTGCTTATTATCACTGCCTTAGTTTTTAGT AGTTTATGGTCATCTGTGTCACCAGATGCTCAAGGGGATGCATTATTTGCGTTGAGGAGC TCGTTACGTGCATCTCCTGAACAGCTTAGTGATTGGAACCAGAATCAAGTCGATCCTTGT ACTTGGTCTCAAGTTATTTGTGATGACAAGAAACATGTTACTTCTGTAACCTTGTCTTAC 15 ATGAACTTCTCCTCGGGAACACTGTCTTCAGGAATAGGAATCTTGACAACTCTCAAGACT CTTACATTGAAGGGAAATGGAATAATGGGTGGAATACCAGAATCCATTGGAAATCTGTCT GGTAATCTCAAGAATCTACAGTTCTTCAGGACCTTGAGTAGGAATAACCTTAATGGTTCT A TCCCGGATTCACTTACAGGTCTATCAAAACTGATAAATATTCTGCTCGACTCAAATAAT 20 CTCAGTGGTGAGATTCCTCAGAGTTTATTCAAAATCCCAAAATACAATTTCACAGCAAAC AACTTGAGCTGTGGCGCACTTTCCCGCAACCTTGTGTAACCGAGTCCAGTCCTTCAGGT GATTCAAGCAGTAGAAAACTGGAATCATCGCTGGAGTTGTTAGCGGAATAGCGGTTATT CTACTAGGATTCTTCTTCTTCTTCTCCCAAGGATAAACATAAAGGATATAAACGAGAC GTATTTGTGGATGTTGCAGGAACGAACTTTAAAAAAGGTTTGATTTCAGGTGAAGTGGAC 25 AGAAGGATTGCTTTTGGACAGTTGAGAAGATTTGCATGGAGAGAGCTTCAGTTGGCTACA GATGAGTTCAGTGAAAAGAATGTTCTCGGACAAGGAGGCTTTGGGAAAGTTTACAAAGGA TTGCTTTCGGATGGCACCAAAGTCGCTGTAAAAAGATTGACTGATTTTGAACGTCCAGGA GGAGATGAAGCTTTCCAGAGAGAAGTTGAGATGATAAGTGTAGCTGTTCATAGGAATCTG CTTCGCCTTATCGGCTTTTGTACAACACAAACTGAACGACTTTTGGTGTATCCTTTCATG 30 CAGAATCTAAGTGTTGCATATTGCTTAAGAGAGATTAAACCCGGGGATCCAGTTCTGGAT TGGTTCAGGAGGAAACAGATTGCGTTAGGTGCAGCACGAGGACTCGAATATCTTCATGAA CATTGCAACCCGAAGATCATACACAGAGATGTGAAAGCTGCAAATGTGTTACTAGATGAA CACTTTCAAGCAGTGGTTGGTGATTTTGGTTTAGCCAAGTTGGTAGATGTTAGAAGGACT AAT GTAACCACTCAGGTCCGAGGAACAATGGGTCATATTGCACCAGAATGTATATCCACA 35 GGGAAATCGTCAGAGAAAACCGATGTTTTCGGGTACGGAATTATGCTTCTGGAGCTTGTA ACTGGACAAAGAGCAATTGATTTCTCGCGGTTAGAGGAAGAAGATGATGTCTTATTGCTA GACCATGTGAAGAAACTGGAAAGAGAGAGAGAGATTAGAAGACATAGTAGATAAGAAGCTT GATGAGGATTATATAAAGGAAGAAGTTGAAATGATGATACAAGTAGCTCTGCTATGCACA CAAGCACCACCGGAAGAACGACCAGCGATGTCGGAAGTAGTAAGAATGCTAGAAGGAGAA 40 GGGCTTGCAGAGAGATGGGAAGAGTGGCAGAATCTTGAAGTGACGAGACAAGAGAGTTT

CAGAGGTTGCAGAGGAGATTTGATTGGGTGAAGATTCCATTAATAATCAAGATGCTATT GAATTATCTGGTGGAAGATAGaaacaaaaaa (SEQ ID NO: 42)

- 5 Predicted amino acid sequence of the Arabidopsis thaliana RKS2 protein.
 - Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).
- 10 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
- bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 3 complete and 2 incomplete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site
- 20 for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
- 25 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions. Italics indicate an alternatively spliced gene
- 30 product.

MALLIITALVFSSL WSSVSPDAGG

35 DALFALRSSLR ASPEOLSDWNONOVD

PCTWSOVICDDKKHVTSV

TLSYMNFSS GTLSSGI

60

G ILTTLKTLTLKGNGIMGGI

PESIGNLSSLTSLDLEDNHLTDRI
5 PSTLGNLKNLQFLTLSRNNLNGSI

PDSLTGLSKLINILLDSNNLSGEI

POSLFKIPKYN FTANNLSCGG

TFPQPCVTESSPSGDSSSRKTG

10

IIAGVVSGIAVIL

LGFFFFFFC

KDKHKGYKRDVFVDVAGTNFKKGLISGE

15 VDRRIAFGOLRRFAWRELOLAT

DEFSEKNVLGQGGFGKVYKGLLSD

GTKVAVKRLTDFERPGGDEAFQ

REVEMISVAVHRNLLRLIGFCT
20 TQTERLLVYPFMQNLSVAYCLR

EIKPGDPVLDWFRRKQIALGAA

RGLEYLHEHCNPKIIHRDVKAA

NVLLDEDFEAVVGDFGLAKLVD VRRTNVTTQVRGTMGHIAPECI

25 STGKSSEKTDVFGYGIMLLELV

TGORAIDFSRLEEEDDVLLLDH

VKKLEREKRLEDIVDKKLDEDY IKEEVEMMIOVALLCTOAAPEE

RPAMSEVVRMLE

30

GEGLAERWEEWQNLEVTRQEEFQ

RLQRRFDWGEDSINNQDAIELSGGR (SEQ ID NO: 43)

35

Arabidopsis thaliana RKS3 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 aacaatcagaaattgatcttacaatgtttcATGGCCTTAGCTTTGTGGGAATCACTTCG TCAACAACTCAACCAGATATCGAAGGAGGAGCTCTGTTGCAGCTCAGAGATTCGCTTAAT GATTCGAGCAATCGTCTAAAATGGACACGCGATTTTGTGAGCCCTTGCTATAGTTGGTCT TATGTTACCTGCAGAGGCCAGAGTGTTGTGGCTCTAAATCTTGCCTCGAGTGGATTCACA GGAACACTCTCCCAGCTATTACAAAACTGAAGTTCTTGGTTACCTTAGAGTTACAGAAC 15 AATAGTTTATCTGGTGCCTTACCAGATTCTCTTGGGAACATGGTTAATCTACAGACTTTA AACCTATCAGTGAATAGTTTCAGCGGATCGATACCAGCGAGCTGGAGTCAGCTCTCGAAT CTAAAGCACTTGGATCTCTCATCCAATAATTTAACAGGAAGCATCCCAACACAATTCTTC TCAATCCCAACATTCGATTTTCAGGAACTCAGCTTATATGCGGTAAAAGTTTGAATCAG CCTTGTTCTTCAAGTTCTCGTCTTCCAGTCACATCCTCCAAGAAAAAGCTGAGAGACATT 20 ACTITGACTGCAAGTTGTGTTGCTTCTATAATCTTATTCCTTGGAGCAATGGTTATGTAT CATCACCATCGCGTCCGCAGAACCAAATACGACATCTTTTTTGATGTAGCTGGGGAAGAT GACAGGAAGATTTCCTTTGGACAACTAAAACGATTCTCTTTACGTGAAATCCAGCTCGCA ACAGATAGTTTCAACGAGAGCAATTTGATAGGACAAGGAGGATTTGGTAAAGTATACAGA GGTTTGCTTCCAGACAAAACAAAAGTTGCAGTGAAACGCCTTGCGGATTACTTCAGTCCT 25 GGAGGAGAAGCTGCTTTCCAAAGAGAGATTCAGCTCATAAGCGTTGCGGTTCATAAAAAT CTCTTACGCCTTATTGGCTTCTGCACAACTTCCTCTGAGAGAATCCTTGTTTATCCATAC GACTGGCCAACAAGGAAGCGTGTAGCTTTTGGTTCAGCTCACGGTTTAGAGTATCTACAC GAACATTGTAACCCGAAGATCATACACCGCGATCTCAAGGCTGCAAACATACTTTTAGAC 30 AACAATTTTGAGCCAGTTCTTGGAGATTTCGGTTTAGCTAAGCTTGTGGACACATCTCTG ACTCATGTCACAACTCAAGTCCGAGGCACAATGGGTCACATTGCGCCAGAGTATCTCTGC ACAGGAAAATCATCTGAAAAAACCGATGTTTTTGGTTACGGTATAACGCTTCTTGAGCTT GTTACTGGTCAGCGCGCAATCGATTTTTCACGCTTGGAAGAAGAGGAAAATATTCTCTTG CTTGATCATATAAAGAAGTTGCTTAGAGAACAGAGACTTAGAGACATTGTTGATAGCAAT 35 TTGACTACATATGACTCCAAAGAAGTTGAAACAATCGTTCAAGTGGCTCTTCTCTGCACA CAAGGCTCACCAGAAGATAGACCAGCGATGTCTGAAGTGGTCAAAATGCTTCAAGGGACT GGTGGTTTGGCTGAGAAATGGACTGAATGGGAACAACTTGAAGAAGTTAGGAACAAAGAA GCATTGTTGCTTCCGACTTTACCGGCTACTTGGGATGAAGAAGAAACCACCGTTGATCAA GAATCTATCCGATTATCGACAGCAAGATGAagaagaaacagagagagaaagatatctatg 40 aaaa (SEQ ID NO: 44)

Predicted amino acid sequence of the Arabidopsis thaliana RKS3 protein.

5 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a

- 10 leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 4 complete repeats of each
- 15 approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for Oglycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular
- 20 domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single
 - leucine rich repeat, probably involved in protein / protein interactions.

MALAFVGITSSTTQPDIEG

30

GALLQLRDSLNDSSNRL KWTRDFVS

PCYSWSYVTCRGOSVVAL

35

NLASSGFTGTLS
P AITKLKFLVTLELQNNSLSGAL
PDSLGNMVNLQTLNLSVNSFSGSI

63

PASWSQLSNLKHLDLSSNNLTGSI PTQFFSIPTFEFSGTQLICGKS

5 LNQPCSSSRLPVTSSKKKLRD

ITLTASCVASIIL FLGAMVMYHHH

10

RVRRTKYDIFFDVAGEDDR KISFGOLKRFSLREIOLAT

DSFNESNLIGQGGFGKVYRGLLPD

15 KTKVAVKRLADYFSPGGEAAFQ

REIQLISVAVHKNLLRLIGFCT

TSSERILVYPYMENLSVAYRLR DLKAGEEGLDWPTRKRVAFGSA

HGLEYLHERCNPKIIHRDLKAA

20 NILLDNNFEPVLGDFGLAKLVD

TSLTHVTTQVRGTMGHIAPEYL

CTGKSSEKTDVFGYGITLLELV

TGQRAIDFSRLEEEENILLLD HIKKLLREQRLRDIVDSNLTTY

25 DSKEVETIVQVALLCTQGSPED

RPAMSEVVKMLQ

GTGGLAEKWTEWEQLEEVRNKEALLL

30 PTLPATWDEEETTVDQESIRLSTAR (SEQ ID NO: 45)

Arabidopsis thaliana RKS4 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters

tcttccttctcggtaatctaatctaaagcttttcATGGTGGTGATGAAGATATTC CCTGAAGTGGAGGCGTTGATAAACATAAAGAACGAGTTACATGATCCACATGGTGTTTTC AAAAACTGGGATGAGTTTTCTGTTGATCCTTGTAGCTGGACTATGATCTCTTGTTCTTCA GACAACCTCGTAATTGGCTTAGGAGCTCCAAGTCAGTCTCTTTCAGGAACTTTATCTGGG 15 TCTATTGGAAATCTCACTAATCTTCGACAAGTGTCATTACAGAACAATAACATCTCCGGT AAAATCCCACCGGAGATTTGTTCTCTTCCCAAATTACAGACTCTGGATTTATCCAATAAC CGGTTCTCCGGTGAAATCCCCGGTTCTGTTAACCAGCTGAGTAATCTCCAATATCTCTTG AACAACAACTCATTATCTGGGCCCTTTCCTGCTTCTCTGTCTCAAATCCCTCACCTCTCT TTCTTAGACTTGTCTTATAACAATCTCAGAGGTCCTGTTCCTAAATTTCCTGCAAGGACA TTCAATGTTGCTGGGAACCCTTTGATTTGTAAAAACAGCCTACCGGAGATTTGTTCAGGA TCAATCAGTGCAAGCCCTCTTTCTGTCTCTTTACGTTCTTCATCAGGACGTAGAACCAAC GGGTTCATTTGGTATCGAAAGAACAAAGACGGTTAACGATGCTTCGCATTAACAAGCAA GAGGAAGGGTTACTTGGGTTGGGAAATCTAAGAAGCTTCACATTCAGGGAACTTCATGTA GCTACGGATGGTTTTAGTTCCAAGAGTATTCTTGGTGCTGGTGGGTTTGGTAATGTCTAC AGAGGAAAATTCGGGGATGGGACAGTGGTGCAGTGAAACGATTGAAAGATGTGAATGGA ACCTCCGGGAACTCACAGTTTCGTACTGAGCTTGAGATGATCAGCTTAGCTGTTCATAGG AATTTGCTTCGGTTAATCGGTTATTGTGCGAGGTTCTAGCGAAAGACTTCTTGTTTACCCT TACATGTCCAATGGCAGCGTCGCCTCTAGGCTCAAAGCTAAGCCAGCGTTGGACTGGAAC 30 ACAAGGAAGAAGATAGCGATTGGAGCTGCAAGAGGGTTGTTTTATCTACACGAGCAATGC GATCCCAAGATTATTCACCGAGATGTCAAGGCAGCAAACATTCTCCTAGATGAGTATTTT GAAGCAGTTGTTGGGGATTTTGGACTAGCAAAGCTACTCAACCACGAGGATTCACATGTC ACAACCGCGGTTAGAGGAACTGTTGGTCACATTGCACCTGAGTATCTCTCCACCGGTCAG TCATCTGAGAAAACCGATGTCTTTGGGTTCGGTATACTTTTGCTAGAGCTCATCACAGGA 35 ATGAGAGCTCTCGAGTTTGGCAAGTCTGTTAGCCAGAAAGGAGCTATGCTAGAATGGGTG AGGAAGCTACACAAGGAAATGAAAGTAGAGGAGCTAGTAGACCGAGAACTGGGGACAACC TACGATAGAATAGAAGTTGGAGAGATGCTACAAGTGGCACTGCTCTGCACTCAGTTTCTT CCAGCTCACAGACCCAAAATGTCTGAAGTAGTTCAGATGCTTGAAGGAGATGGATTAGCT GAGAGATGGGCTGCTTCACATGACCATTCACATTTCTACCATGCCAACATGTCTTACAGG 40 ACTATTACCTCTACTGATGGCAACCAAACCAAACCACCTCTGTTTGGCTCCTCAGGATTT

GAAGATGAAGATGATAATCAAGCGTTAGATTCATTCGCCATGGAACTATCTGGTCCAAGG

<u>TAG</u>taaatcttggacacagaaagaaacagatataatatccccatgacttcaatttttgtt (SEQ ID NO: 46)

- 5 Predicted amino acid sequence of the Arabidopsis thaliana RKS4 protein.
 - Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).
- 10 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 2 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
- bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for 0-
- 20 glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
- 25 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MVVMKLITMKIFSVLLLL CFFVTCSLSSEPRNPEV

EALINIKNELHDP

35 HGVFKNWDEFSVD

PCSWTMISCSSDNLVIGL

GAPSOSLSGTLS

G SIGNLTNLRQVSLONNNISGKI

PPEICSLPKLQTLDLSNNRFSGE1

PGSVNQLSNLQYLRLNNNSLSGPF

5 PASLSQIPHLSFLDLSYNNLRGPV

PKFPARTFNVAGNPLICKNS

LPEICSGSISASPL

SVSLRSSSGRRTN

10

ILAVALGVSLGFAVSVIL

SLGFIWY

RKKORRLTMLRINKOEE

15 GLLGLGNLRSFTFRELHVAT

DGFSSKSILGAGGFGNVYRGKFGD

GTVVAVKRLKDVNGTSGNSQFR

TELEMISLAVHRNLLRLIGYCA 20 SSSERLLVYPYMSNGSVASRLK

AKPALDWNTRKKIAIGAA

RGLFYLHEQCDPKIIHRDVKAA

NILLDEYFEAVVGDFGLAKLLN

HEDSHVTTAVRGTVGHIAPEYL

25 STGQSSEKTDVFGFGILLLELI

TGMRALEFGKSVSQKGAMLEW VRKLHKEMKVEELVDRELGTTY

DRIEVGEMLQVALLCTQFLPAH

RPKMSEVVOMLE

30

GDGLAERWAASHDHSHFYHANM

SYRTITSTDGNNQTKHLFG

SSGFEDEDDNQALDSFAMELSGPR (SEQ ID NO: 47)

35

Arabidopsis thaliana RKS5 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

ctaqaqaattcttatactttttctacqATGGAGATTTCTTTGATGAAGTTTCTGTTTTTA GGAATCTGGGTTTATTATTACTCTGTTCTTGACTCTGTTTCTGCCATGGATAGTCTTTTA TCTCCCAAGGTGGCTGCGTTAATGTCAGTGAAGAACAAGATGAAAAGATGAGAAAGAGGGTT TCTGAAGGTTTTGTGGTTTCTCTAGAGATGGCTAGTAAAGGATTATCAGGGATACTATCT ACTAGTATTGGGGAATTAACTCATCTTCATACTTTGTTACTTCAGAATAATCAGTTAACT GGTCCGATTCCTTCTGAGTTAGGCCAACTCTCTGAGCTTGAAACGCTTGATTTATCGGGG AATCGGTTTAGTGGTGAAATCCCAGCTTCTTTAGGGTTCTTAACTCACTTAAACTACTTG CGGCTTAGCAGGAATCTTTTATCTGGGCAAGTCCCTCACCTCGTCGCTGGCCTCTCAGGT CTTTCTTTCTTGGATCTATCTTTCAACAATCTAAGCGGACCAACTCCGAATATATCAGCA AAAGATTACAGGAAATGCATTTCTTTGTGGTCCAGCTTCCCAAGAGCTTTGCTCAGATGC TACACCTGTGAGAAATGCTGCAATCGATCTGCAGCGACGGGTTTGTCTGAAAAGGACAAT AGCAAACATCACAGCTTAGTGCTCTCTTTTGCATTTGGCATTGTTGTTGTTGCCTTTATCATC TCCCTAATGTTTCTCTTCTTCTGGGTGCTTTGGCATCGATCACGTCTCTCAAGATCACAC GTGCAGCAAGACTACGAATTTGAAATCGGCCATCTGAAAAGGTTCAGTTTTCGCGAAATA CAAACCGCAACAAGCAATTTTAGTCCAAAGAACATTTTGGGACAAGGAGGGTTTGGGATG GTTTATAAAGGGTATCTCCCAAATGGAACTGTGGTGGCAGTTAAAAGATTGAAAGATCCG ATTTATACAGGAGAAGTTCAGTTTCAAACCGAAGTAGAGATGATTGGCTTAGCTGTTCAC CGTAACCTTTTACGCCTCTTTGGATTCTGTATGACCCCGGAAGAGAGAATGCTTGTGTAT CCGTACATGCCAAATGGAAGCGTAGCTGATCGTCTGAGAGATTGGAATCGGAGGATAAGC 30 ATTGCACTCGGCGCAGCTCGAGGACTTGTTTACTTGCACGAGCAATGCAATCCAAAGATT ATTCACAGAGACGTCAAAGCTGCAAATATTCTACTTGATGAGAGCCTTTGAAGCAATAGTT GGCGATTTTGGTCTAGCAAAGCTTTTAGACCAGAGAGATTCACATGTCACTACCGCAGTC CGAGGAACCATTGGACACATCGCTCCCGAGTACCTTTCCACTGGACAGTCCTCAGAGAAA ACCGATGTTTTCGGATTCGGAGTACTAATCCTTGAACTCATAACAGGTCATAAGATGATT 35 CATCAAGGCAATGGTCAAGTTCGAAAAGGAATGATATTGAGCTGGGTAAGGACATTGAAA GCAGAGAGAGAGTTTGCAGAGATGGTGGACAGAGATTTGAAGGGAGAGTTTGATGATTTG GTGTTGGAGGAAGTAGTGGAATTGGCTTTGCTTTGTACACAGCCACATCCGAATCTAAGA CCGAGGATGTCTCAAGTGTTGAAGGTACTAGAAGGTTTAGTGGAACAGTGTGAAGGAGGG TATGAAGCTAGAGCTCCAAGTGTCTCTAGGAACTACAGTAATGGTCATGAAGAGCAGTCC 40 TTTATTGAAGCCATTGAGCTCTCTGGACCACGATGAtagacttcatagtgtcttaac

tagtcttcttgattttgttgtcattgtcatggc (SEQ ID NO: 48)

Predicted amino acid sequence of the Arabidopsis thaliana RKS5 protein.

5 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains no

- 10 leucine zipper motif, in contrast to the other RKS proteins. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues.
- 15 The fifth domain contains many serine residues, and is likely to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine /
- 20 threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / 25 protein interactions.

MEISLMKFLFLGIWVYYYS VLDSVSAMDSILSPKV

30

AALMSVKNKMKDE KEVLSGWDINSVD

PCTWNMVGCSSEGFVVS

35

LEMASKGLSGILS
T SIGELTHLHTLLLQNNQLTGPI
PSELGQLSELETLDLSGNRFSGEI
PASLGFLTHLNYLRLSGNVLSGOV

WO 2004/007712

PCT/NL2003/000524

PHLVAGLSGLSFLDLSFNNLSGPT PNISAK DYRKCISLWSSFPR 69

ALLRCYTCEKCCNR

5 SAATGLSEKDNSK

HHSLVLSFAFGIVV AFIISLMFLFFWVLWH

10 RSRLSRSHVQQDYEF EIGHLKRFSFREIQTAT

GTVVAVKRLKDEIYTGEVQFQ
15 TEVEMIGLAVERKULRUFGFCM
TPEERMLVYFYMPNGSVADRLR
DWNRRISIALGAA
RGLVYLHEQCNPKLIHRDVKAA
NILLDESFEAIVGDFGLAKLLD
20 QRDSWYTTAVKGTIGHTAPEYL
STGQSSEKTDVFGFGVLLLELI
TGKKRIDOGNOVRKGKILSN

VRTLKAEKRFAEMVDRDLKGEF DDLVLEEVVELALLCTQPHPNL

SNFSPKNILGOGGFGMVYKGYLPN

25 RPRMSQVLKV

LEGLVEOCEGGYEARA

PASVSRNYSNGHEEQSFIIEAIELSGPR (SEQ ID NO: 49)

30

Arabidopsis thaliana RKS6 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals. The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

attgtttccttcttttgggattttctccttggatggaaccagctcaattaatgagatgag

10 ATGAGAATGTTCAGCTTGCAGAAGATGGCTATGGCTTTTACTCTCTTGTTTTTTGCCTGT TTATGCTCATTTGTGTCTCCAGATGCTCAAGGGGATGCACTGTTTGCGTTGAGGATCTCC TTACGTGCATTACCGAATCAGCTAAGTGACTGGAATCAGAACCAAGTTAATCCTTGCACT TGGTCCCAAGTTATTTGTGATGACAAAAACTTTGTCACTTCTCTTACATTGTCAGATATG AACTTCTCGGGAACCTTGTCTTCAAGAGTAGGAATCCTAGAAAATCTCAAGACTCTTACT 15 TTAAAGGGAAATGGAATTACGGGTGAAATACCAGAAGACTTTGGAAATCTGACTAGCTTG ACTAGTTTGGATTTGGAGGACAATCAGCTAACTGGTCGTATACCATCCACTATCGGTAAT CTCAAGAAACTTCAGTTCTTGACCTTGAGTAGGAACAAACTTAATGGGACTATTCCGGAG TCACTCACTGGTCTTCCAAACCTGTTAAACCTGCTGCTTGATTCCAATAGTCTCAGTGGT CAGATTCCTCAAAGTCTGTTTGAGATCCCAAAATATAATTTCACGTCAAACAACTTGAAT 20 TGTGGCGGTCGTCAACCTCACCCTTGTGTATCCGCGGTTGCCCCATTCAGGTGATTCAAGC AAGCCTAAAACTGGCATTATTGCTGGAGTTGTTGCTGGAGTTACAGTTGTTCTCTTTGGA ATCTTGTTGTTCTGTTCTGCAAGGATAGGCATAAAGGATATAGACGTGATGTTTTGTG CATGTTGCAGGTGAAGTGGACAGGAGAATTGCATTTGGACAGTTGAAAAGGTTTGCATGG AGAGAGCTCCAGTTAGCGACAGATAACTTCAGCGAAAAGAATGTACTTGGTCAAGGAGGC 25 TTTGGGAAGTTTACAAAGGAGTGCTTCCGGATACACCCAAAGTTGCTGTGAAGAGATTG ACGGATTTCGAAAGTCCTGGTGGAGATGCTGCTTTCCAAAGGGAAGTAGAGATGATAAGT CARGAGACCACCACTTAGATTGGGAGACGAGGAAACGGATTGCCTTAGGAGCAGCGCGT 30 GGTTTTGAGTATCTTCATGAACATTGCAATCCGAAGATCATACATCGTGATGTGAAAGCA CTAGTAGATGTTAGAAGGACTAATGTGACTACTCAAGTTCGAGGAACAATGGGTCACATT GCACCAGAATATTTATCAACAGGGAAATCATCAGAGAGAACCGATGTTTTCGGGTATGGA ATTATGCTTCTTGAGCTTGTTACAGGACAACGCGCAATAGACTTTTCACGTTTGGAGGAA GTGAGGATGTTAGAAGGAGAGGGCTTGCGGAGAGATGGGAAGAGTGGCAAAACGTGGAA CTCACCACACCTCATGAGTTTGAACGGTTGCAGAGGAGATTTGATTGGGGTGAAGATTCT ATGCATAACCAAGATGCCATTGAATTATCTGGTGGAAGATGACCaaaaacatcaaacctt (SEQ ID NO: 50) 71

Predicted amino acid sequence of the Arabidopsis thaliana RKS6 protein.

Different domains are spaced and shown from the N-terminus

- 5 towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).
 - At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each
- separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain
- 15 contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for Oglycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown
- 20 function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single
- 25 leucine rich repeat, probably involved in protein / protein interactions.

MRMFSL

QKMAMAFTLLFFACLCSFVSPDAGG

30

DALFALRISLRALP NQLSDWNQNQVN

PCTWSQVICDDKNFVTSL

35

TLSDMNFSGTLSSRV GILENLKTLTLKGNGITGEI PEDFGNLTSLTSLDLEDNQLTGRI

PSTIGNLKKLQFLTLSRNKLNGTI PESLTGLPNLLNLLLDSNSLSGQI PQSLFEIPKYNFTSNNLNCGG

5 ROPHPCVSAVAHSGDSSKPKTG

IIAGVVAGVTVVL FGILLFLFC

10 KDRHKGYRRDVFVDVAGE

VDRRIAFGQLKRFAWRELQLAT

DNFSEKNVLGQGGFGKVYKGVLPD

TPKVAVKRLTDFESPGGDAAFQ

15 REVEMISVAVHRNLLRLIGFCT

TOTERLLVYPFMQNLSLAHRLR

EIKAGDPVLDWETRKRIALGAA

RGFEYLHEHCNPKIIHRDVKAA NVLLDEDFEAVVGDFGLAKLVD

20 VRRTNYTTQVRGTMGHIAPEYL

STGKSSERTDVFGYGIMLLELV

TGQRAIDFSRLEEEDDVLLLDH

VKKLEREKRLGAIVDKNLDGEY

IKEEVEMMIQVALLCTQGSPED
25 RPVMSEVVRMLE

GEGLAERWEEWONVEVTRRHEFE

RLQRRFDWGEDSMHNQDATELSGGR (SEQ ID NO: 51)

30

Arabidopsis thaliana RKS7 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

acatcttgttttctgctcattcctctgtttcaacaATGGAGAGTACTATTGTTATGATGA TGATGATAACAAGATCTTTCTTTTGCTTCTTGGGATTTTTATGCCTTCTCTGCTCTTCTG TTCACGGATTGCTTTCTCCTAAAGGTGTTAACTTTGAAGTGCAAGCTTTGATGGACATAA AAGCTTCATTACATGATCCTCATGGTGTTCTTGATAACTGGGATAGAGATGCTGTTGATC CTTGTAGTTGGACAATGGTCACTTGTTCTTCTGAAAACTTTGTCATTGGCTTAGGCACAC 15 CAAGTCAGAATTTATCTGGTACACTATCTCCAAGCATTACCAACTTAACAAATCTTCGGA TTGTGCTGTTGCAGAACAACAACATAAAAGGAAAAATTCCTGCTGAGATTGGTCGGCTTA CGAGGCTTGAGACTCTTGATCTTTCTGATAATTTCTTCCACGGTGAAATTCCTTTTTCAG TTCCTCTGTCACTATCTAATATGACTCAACTTGCCTTTCTTGATTTATCATACAACAATC 20 TTAGTGGTCCTGTTCCAAGATTTGCTGCAAAGACGTTTAGCATCGTTGGGAACCCGCTGA TATGTCCAACGGGTACCGAACCAGACTGCAATGGAACAACATTGATACCTATGTCTATGA ACTTGAATCAAACTGGAGTTCCTTTATACGCCGGTGGATCGAGGAATCACAAAATGGCAA TCTGGTGGAGACAAAGACATAACCAAAACACATTCTTTGATGTTAAAGATGGGAATCATC 25 ATGAGGAAGTTTCACTTGGAAACCTGAGGAGATTTGGTTTCAGGGAGCTTCAGATTGCGA CCAATAACTTCAGCAGTAAGAACTTATTGGGGAAAGGTGGCTATGGAAATGTATACAAAG GAATACTTGGAGATAGTACAGTGGTTGCAGTGAAAAGGCTTAAAGATGGAGGAGCATTGG GAGGAGAGATTCAGTTTCAGACAGAAGTTGAAATGATCAGTTTAGCTGTTCATCGAAATC TCTTAAGACTCTACGGTTTCTGCATCACACAAACTGAGAAGCTTCTAGTTTATCCTTATA 30 TGTCTAATGGAAGCGTTGCATCTCGAATGAAAGCAAAACCTGTTCTTGACTGGAGCATAA GGAAGAGGATAGCCATAGGAGCTGCAAGAGGGCTTGTGTATCTCCATGAGCAATGTGATC CGAAGATTATCCACCGCGATGTCAAAGCAGCGAATATACTTCTTGATGACTACTGTGAAG $\tt CTGTGGTTGGCGATTTTGGTTTAGCTAAACTCTTGGATCATCAAGATTCTCATGTGACAA$ CCGCGGTTAGAGGCACGGTGGGTCACATTGCTCCAGAGTATCTCTCAACTGGTCAATCCT 35 CTGAGAAAACAGATGTTTTTGGCTTCGGGATTCTTCTTCTTGAGCTTGTAACCGGACAAA GAGCTTTTGAGTTTGGTAAAGCGGCTAACCAGAAAGGTGTGATGCTTGATTGGGTTAAAA AGATTCATCAAGAGAAGAAACTTGAGCTACTTGTGGATAAAGAGTTGTTGAAGAAGAAGA GCTACGATGAGATTGAGTTAGACGAAATGGTAAGAGTAGCTTTGTTGTGCACACAGTACC TGCCAGGACATAGACCAAAAATGTCTGAAGTTGTTCGAATGCTGGAAGGAGGAGATGGACTTG CAGAGAAATGGGAAGCTTCTCAAAGATCAGACAGTGTTTCAAAATGTAGCAACAGGATAA

ATGAATTGATGTCATCTTAGACAGATACTCTGATCTTACCGATGACTCTAGTTTACTTG
TGCAAGCAATGGAGCTCTCTGGTCCTAGA<u>TGA</u>aatctatacatgaatctgaagaagaaga agaacatgcatctgtttcttgaatcaagagggattcttgttttttgtataatagagagg ttttttggagggaaatgttgtgtctctgtaactgtataggcttgttgtgtaagaagttat

10 Predicted amino acid sequence of the Arabidopsis thaliana RKS7 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

15 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate

20 bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for 0-

25 glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably

30 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

MESTIVMMMMITRSFF CFLGFLCLLCSSVHGLLSPKGVNFEV

35

QALMDIKASLHDP HGVLDNWDRDAVD

PCSWTMVTCSSENFVIG

5

SPSITNLTNLRIVLLQNNNIKGKI
PAEIGRLTRLETLDLSDNFFHGEI
PFSVGYLQSLQYLRLNNNSLSGVF
10 PLSLSMMTQLAFLDLSYNNLSGFV
PRFAA KTFSIVGNPLICPT

LGTPSONLSGTL

GTEPDCNGTTLIPMSMNL NOTGVPLYAGGSRNHKMA

15
IAVGSSVGTVSLIFIAVGLFLWW

RQRHNQNTFFDVKDGNHHE EVSLGNLRRFGFRELQIAT

20

NNFSSKNLLGKGGYGNVYKGLIGD
STVVAVKRLENGGALGGEIGPQ
TEVEMISLAVHRNLLRLYGFCI
TQTEKLLVYFYMSNGSVA
25 SRMKAKPVLDWSIRKRIAIGAA
RGLVYLHEQCDPKTIHRDVKAA
NILLDDYCEAVVGDFGLAKLLD
HQDSHVTTAVRGTVGHIAPEVL
STGQSSEKTDVFGFGILLLELV
VKXTHQERKLELLVDKELLKKKSY
DEILDEMVRVALLCTQYLPGH
RPKNSEVVRNLE

35 GDGLAEKWEASQRSDS VSKCSNRINELMSSS

DRYSDLTDDSSLLVQAMELSGPR (SEQ ID NO: 53)

Arabidopsis thaliana RKS8 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

- 5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.
- 10 qttttttttttttttaccctcttggaggatctgggaggagaaatttqcttttttttggtaa ATGGGGAGAAAAAGTTTGAAGCTTTTGGTTTTGTCTGCTTAATCTCACTGCTTCTTCTG TTTAATTCGTTATGGCTTGCCTCTTCTAACATGGAAGGTGATGCACTGCACAGTTTGAGA GCTAATCTAGTTGATCCAAATAATGTCTTGCAAAGCTGGGATCCTACGCTTGTTAATCCG TGTACTTGGTTTCACGTAACGTGTAACAACGAGAACAGTGTTATAAGAGTCGATCTTGGG 15 AATGCAGACTTGTCTGGTCAGTTGGTTCCTCAGCTAGGTCAGCTCAAGAACTTGCAGTAC TTGGAGCTTTATAGTAATAACATAACCGGGCCGGTTCCAAGCGATCTTGGGAATCTGACA AACTTAGTGAGCTTGGATCTTTACTTGAACAGCTTCACTGGTCCAATTCCAGATTCTCTA GGAAAGCTATTCAAGCTTCGCTTTCTTCGGCTCAACAATAACAGTCTCACCGGACCAATT CCCATGTCATTGACTAATATCATGACCCTTCAAGTTTTGGATCTGTCGAACAACCGATTA 20 TCCGGATCTGTTCCTGATAATGGTTCCTTCTCGCTCTTCACTCCCATCAGTTTTGCTAAC AACTTGGATCTATGCGGCCCAGTTACTAGCCGTCCTTGTCCTGGATCTCCCCCGTTTTCT CCTCCACCACCTTTATACCACCTCCCATAGTTCCTACACCAGGTGGGTATAGTGCTACT GGAGCCATTGCGGGAGGAGTTGCTGCTGGTGCTGCTTTACTATTTGCTGCCCCTGCTTTA GCTTTTGCTTGGTGGCGTAGAAGAAAACCTCAAGAATTCTTCTTTGATGTTCCTGCCGAA 25 GAGGACCCTGAGGTTCACTTGGGGCAGCTTAAGCGGTTCTCTCTACGGGAACTTCAAGTA GCAACTGATAGCTTCAGCAACAAGAACATTTTGGGCCGAGGTGGGTTCGGAAAAGTCTAC AAAGGCCGTCTTGCTGATGGAACACTTGTTGCAGTCAAACGGCTTAAAGAAGAGCGAACC CCAGGTGGCGAGCTCCAGTTTCAGACAGAAGTGGAGATGATAAGCATGGCCGTTCACAGA AATCTCCTCAGGCTACGCGGTTTCTGTATGACCCCTACCGAGAGATTGCTTGTTTATCCT 30 TACATGGCTAATGGAAGTGTCGCTTCCTGTTTGAGAGAACGTCCACCATCACAGTTGCCT CTAGCCTGGTCAATAAGACAGCAAATCGCGCTAGGATCAGCGAGGGGTTTGTCTTATCTT CATGATCATTGCGACCCCAAAATTATTCACCGTGATGTGAAAGCTGCTAATATTCTGTTG GACGAGGAATTTGAGGCGGTGGTAGGTGATTTCGGGTTAGCTAGACTTATGGACTATAAA GATACTCATGTCACAACGGCTGTGCGTGGGACTATTGGACACATTGCTCCTGAGTATCTC 35 TCAACTGGAAAATCTTCAGAGAAAACTGATGTTTTTGGCTACGGGATCATGCTTTTGGAA CTGATTACAGGTCAGAGAGCTTTTGATCTTGCAAGACTGGCGAATGACGATGACGTTATG CTCCTAGATTGGGTGAAAGGGCTTTTGAAGGAGAAGAAGCTGGAGATGCTTGTGGATCCT GACCTGCAAAGCAATTACACAGAAGCAGAAGTAGAACAGCTCATACAAGTGGCTCTTCTC TGCACACAGAGCTCACCTATGGAACGACCTAAGATGTCTGAGGTTGTTCGAATGCTTGAA 40 GGTGACGGTTTAGCGGAGAAATGGGACGACTGGCAGAAAGTGGAAGTTCTCAGGCAAGAA

GTGGAGCTCTCTCTCACCCCACCTCTGACTGGATCCTTGATTCGACTGATAATCTTCAT GCTATGGAGTTGTCTGGTCCAAGA<u>TAA</u>acgacattgtaatttgcctaacagaaaagagaa agaacagagaaatattaagagaatcacttctctgtattctt (SEQ ID NO: 54)

5

Predicted amino acid sequence of the Arabidopsis thaliana RKS8 protein.

Different domains are spaced and shown from the N-terminus

10 towards the C-terminus. Overall domain structure is similar as
described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing i leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline

20 residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30 MGRKKFEAFGFVCLISLLLLFNSL WLASSNMEG

> DALHSLRANLVDP NNVLQSWDPTLVN

35

PCTWFHVTCNNENSVIRV

DLGNADLSGQLV
P QLGQLKNLQYLELYSNNITGPV
40 PSDLGNLTNLVSLDLYLNSFTGPI

PDSLGKLFKLRFLRLNNNSLTGPI PMSLTNIMTLQVLDLSNNRLSGSV PDNGSFSLFTPISFANNLDLCGPV 78

5 TSRPCPGSPPFSPPPP FIPPPIVPTPGGYSATG

AIAGGVAAGAAL

LFAAPALAFAWW

10

RRRKPQEFFFDVPAEEDPE VHLGQLKRFSLRELQVAT

DSFSNKNILGRGGFGKVYKGRLAD

15 GTLVAVKRLKEERTPGGELQFQ TEVEMISMAVHRNLLRLRGFCM

> TPTERLLVYPYMANGSVASCLR ERPPSOLPLAWSIRQQIALGSA

RGLSYLHDHCDPKIIHRDVKAA

20 NILLDEEFEAVVGDFGLARLMD

YKDTHVTTAVRGTIGHIAPEYL

STGKSSEKTDVFGYGIMLLELI TGQRAFDLARLANDDDVMLLDW

VKGLLKEKKLEMLVDPDLQSNY

25 TEAEVEQLIQVALLCTQSSPME RPKMSEVVRMLE

GDGLAEKWDEWQKVEVLRQEVELS

30 SHPTSDWILDSTDNLHAMELSGPR (SEQ ID NO: 55)

Arabidopsis thaliana rks10 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

taatctcttgaggataaaATGGAACGAAGATTAATGATCCCTTGCTTCTTTTGGTTGATT CTCGTTTTGGATTTGGTTCTCAGAGTCTCGGGCAACGCCGAAGGTGATGCTCTAAGTGCA CTGAAAAACAGTTTAGCCGACCCTAATAAGGTGCTTCAAAGTTGGGATGCTACTCTTGTT ACTCCATGTACATGGTTTCATGTTACTTGCAATAGCGACAATAGTGTTACACGTGTTGAC 15 CTTGGGAATGCAAATCTATCTGGACAGCTCGTAATGCAACTTGGTCAGCTTCCAAACTTG CAGTACTTGGAGCTTTATAGCAATAACATTACTGGGACAATCCCAGAACAGCTTGGAAAT CTGACGGAATTGGTGAGCTTGGATCTTTACTTGAACAATTTAAGCGGGCCTATTCCATCA ACTCTCGGCCGACTTAAGAAACTCCGTTTCTTGCGTCTTAATAACAATAGCTTATCTGGA GARATTCCARGGTCTTTGACTGCTCTCCTGACGCTACAAGTTCTGGATCTCTCAAACAAT 20 CCTCTCACCGGAGATATTCCTGTTAATGGTTCCTTTTCACTTTTCACTCCAATCAGTTTT GCCAACACCAAGTTGACTCCCCTTCCTGCATCTCCACCGCCTCCTATCTCTCCTACACCG CONTONOCTGORGGROUNDATAGARTTACTGGRGCGATTGCGGGGGGGGGTTGCTGCAGGT GCTGCACTTCTATTTGCTGTTCCGGCCATTGCACTAGCTTGGTGGCGAAGGAAAAAGCCG CAGGACCACTTCTTTGATGTACCAGCTGAAGAGGACCCAGAAGTTCATTTAGGACAACTG 25 AAGAGGTTTTCATTGCGTGAACTACAAGTTGCTTCGGATAATTTTAGCAACAAGAACATA TTGGGTAGAGGTGGTTTTGGTAAAGTTTATAAAGGACGGTTAGCTGATGGTACTTTAGTG GTTGAGATGATTAGTATGGCGGTTCACAGAAACTTGCTTCGGCTTCGTGGATTTTGCATG ACTCCAACCGAAGATTGCTTGTTTATCCCTACATGGCTAATGGAAGTGTTGCCTCCTGT 30 TTAGG GAACGTCCCGAGTCCCAGCCACCTGATTGGCCAAAGAGACAGCGTATTGCG TTGGGATCTGCAAGAGGGCTTGCGTATTTACATGATCATTGCGACCCAAAGATTATTCAT CGAGATGTGAAAGCTGCAAATATTTTGTTGGATGAAGAGTTTGAAGCCGTGGTTGGGGAT TTTGGACTTGCAAAACTCATGGACTACAAAGACACACATGTGACAACCGCAGTGCGTGGG ACAATTGGTCATATAGCCCCTGAGTACCTTTCCACTGGAAAATCATCAGAGAAAACCGAT 35 GTCTTTGGGTATGGAGTCATGCTTCTTGAGCTTATCACTGGACAAAGGGCTTTTGATCTT GCTCGCCTCGCGAATGATGATGTCATGTTACTAGACTGGGTGAAAGGGTTGTTAAAA GAGAAGAATTGGAAGCACTAGTAGATGTTGATCTTCAGGGTAATTACAAAGACGAAGAA GTGGAGCAGCTAATCCAAGTGGCTTTACTCTGCACTCAGAGTTCACCAATGGAAAGACCC AAAATGTCTGAAGTTGTAAGAATGCTTGAAGGAGGTGTTTAGCTGAGAGATGGGAAGAG TGGCAAAAGGAGGAAATGTTCAGACAAGATTTCAACTACCCAACCCACCATCCAGCCGTG

5

Predicted amino acid sequence of the Arabidopsis thaliana RKS10 protein.

Different domains are spaced and shown from the N-terminus 0 towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also 25 containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-

terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30 MERRLMIPCFFWLILVL DLVLRVSGNAEG

> DALSALKNSLADP NKVLOSWDATLVT

35

PCTWFHVTCNSDNSVTRV

DLGNANLSGQLV

M QLGQLPNLQYLELYSNNITGTI 40 PEQLGNLTELVSLDLYLNNLSGPI

PCT/NL2003/000524

PSTLGRLKKLRFLRLNNNSLSGEI

81

PRSLTAVLTLOVLDLSNNPLTGDI

PVNGSFSLTPISFANTK LT PL

5 PASPPPPISPTPPSPAGSNRITG

AIAGGVAAGAAL LFAVPAIALAWW

10 RRKKPQDHFFDVPAEEDPE

VHLGQLKRFSLRELQVAS

DNFSNKNILGRGGFGKVYKGRLAD

GTLVAVKRLKEERTQGGELQFQ

15 TEVEMISMAVHRNLLRLRGFCM

TPTERLLVYPYMANGSVASCLR

ERPESQPPLDWPKRQRIALGSA

RGLAYLHDHCDPKIIHRDVKAA NILLDEEFEAVVGDFGLAKLMD

20 YKDTHVTTAVRGTIGHIAPEYL

STGKSSEKTDVFGYGVMLLELI

TGQRAFDLARLANDDDVMLLDW

VKGLLKEKKLEALVDVDLQGNY KDEEVEOLIOVALLCTOSSPME

25 RPKMSEVVRMLE

GDGLAERWEEWQKEEMFRQDFNYPTHH

PAVSGWIIGDSTSQIENEYPSGPR (SEQ ID NO: 57)

30

Arabidopsis thaliana RKS 11 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

ttgttaacctctcgtaactaaaatcttccATGGTAGTAGTAACAAAGAAGACCATGAAGA

TTCAAATTCATCTCCTTTACTCGTTCTTGTTCCTCTGTTTCTCTACTCTCACTCTATCTT CTGAGCCCAGAAACCCTGAAGTTGAGGCGTTGATAAGTATAAGGAACAATTTGCATGATC CTCATGGAGCTTTGAACAATTGGGACGAGTTTTCAGTTGATCCTTGTAGCTGGGCTATGA 15 GAGGTTTATCTGAGTCTATCGGAAATCTCACAAATCTCCGACAAGTGTCATTGCAAAATA ACAACATCTCCGGCAAAATTCCACCGGAGCTCGGTTTTCTACCCAAATTACAAACCTTGG ATCTTTCCAACAACCGATTCTCCGGTGACATCCCTGTTTCCATCGACCAGCTAAGCAGCC TTCAATATCTGAGACTCAACAACACTCTTTGTCTGGGCCCTTCCCTGCTTCTTTGTCCC AAATTCCTCACCTCTCCTTCTTGGACTTGTCTTACAACAATCTCAGTGGCCCTGTTCCTA 20 AATTCCCAGCAAGGACTTTAAACGTTGCTGGTAATCCTTTGATTTGTAGAAGCAACCCAC CTGAGATTTGTTCTGGATCAATCAATGCAAGTCCACTTTCTGTTTCTTTGAGCTCTTCAT CAGGACGCAGGTCTAATAGATTGGCAATAGCTCTTAGTGTAAGCCTTGGCTCTGTTGTTA TACTAGTCCTTGCTCTCGGGTCCTTTTGTTGGTACCGAAAGAACAAAGAAGACTACTGA TCCTTAACTTAAACGCAGATAAACAAGAGGAAGGGCTTCAAGGACTTGGGAATCTAAGAA GCTTCACATTCAGAGAACTCCATGTTTATACAGATGGTTTCAGTTCCAAGAACATTCTCG GCGCTGGTGGATTCGGTAATGTGTACAGAGGCAAGCTTGGAGATGGGACAATGGTGGCAG TGAAACGGTTGAAGGATATTAATGGAACCTCAGGGGATTCACAGTTTCGTATGGAGCTAG AGATGATTAGCTTAGCTGTTCATAAGAATCTGCTTCGGTTAATTGGTTATTGCGCAACTT CTGGTGAAAGGCTTCTTGTTTACCCTTACATGCCTAATGGAAGCGTCGCCTCTAAGCTTA 30 AATCTAAACCGGCATTGGACTGGAACATGAGGAAGAGGATAGCAATTGGTGCAGCGAGAG CTAATATTCTCTTAGACGAGTGCTTTGAAGCTGTTGTTGGTGACTTTGGACTCGCAAAGC TCCTTAACCATGCGGATTCTCATGTCACAACTGCGGTCCGTGGTACGGTTGGCCACATTG CACCTGAATATCTCTCCACTGGTCAGTCTTCTGAGAAAACCGATGTGTTTGGGTTCGGTA TACTATTGCTCGAGCTCATAACCGGACTGAGAGCTCTTGAGTTTGGTAAAACCGTTAGCC AGAAAGGAGCTATGCTTGAATGGGTGAGGAAATTACATGAAGAGATGAAAGTAGAGGAAC TATTGGATCGAGAACTCGGAACTAACTACGATAAGATTGAAGTTGGAGAGATGTTGCAAG TGGCTTTGCTATGCACACACATATCTGCCAGCTCATCGTCCTAAAATGTCTGAAGTTGTTT TGATGCTTGAAGGCGATGGATTAGCCGAGAGATGGGCTGCTTCGCATAACCATTCACATT TCTACCATGCCAATATCTCTTTCAAGACAATCTCTTCTCTGTCTACTACTTCTGTCTCAA

GSCTTGACGCACATTGCAATGATCCAACTTATCAAATGTTTGGATCTTCGGCTTTCGATG
ATGACGATGATCATCAGCCTTTAGATTCCTTTGCCATGGAACTATCCGGTCCAAGA<u>TAA</u>c
acaatgaaagaagatatcatttttacgatggatcaaacaatccaatgaaaaaa (SEQ ID NO: 58)

5

Predicted amino acid sequence of the Arabidopsis thaliana RKS11 protein.

10 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997). At the predicted extracellular domain the first domain

represents a signal sequence. The second domain contains a
15 leucine zipper motif, containing 3 leucine residues, each
separated by seven other amino acids. The third domain
contains conserved cysteine residues, involved in disulphate
bridge formation. The fourth domain contains a leucine rich

repeat domain, consisting of 5 complete repeats of each
20 approximately 24 amino acid residues. The fifth domain
contains many serine and proline residues, and is likely to
contain hydroxy-proline residues, and to be a site for Oglycosylation. The sixth domain contains a single
transmembrane domain after which the predicted intracellular

25 domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single

leucine rich repeat, probably involved in protein / protein interactions.

MVVVTKKTMKIQIHLLYSFLFL

35 CFSTLTLSSEPRNPEV

EALISIRNNLHDP HGALNNWDEFSVD

PCSWAMITCSPDNLVIGL

GAPSOSLSGGLS

5 ESIGNLTNLRQVSLQNNNISGKI

PPELGFLPKLQTLDLSNNRFSGDI

PVSIDQLSSLQYLRLNNNSLSGPF

PASISQIPHLSFLDLSYNNLSGPV

PKFPARTFNVAGNPLICRSN

10

PPEICSGSINASPL SVSLSSSSGRRSNR

LAIALSVSLGSVVIL
15 VLALGSFCWY

RKKQRRLLILNLNGADKQEE GLQGLGNLRSFTFRELHVYT

20 DGFSSKNILGAGGFGNVYRGKLGD
GTMVAVKRLKDINGTSGDSQFR
MELEMISLAVHKNLLRLIGYCA
TSGERLLVYPYMPNGSVASKLK
SKPALDWNMRKRIAIGAA

25 RGLLYLHEQCDPKIIHRDVKAA NILLDECFEAVVGDFGLAKLLN HADSHVTTAVRGTVGHIAPEYL STGQSSEKTDVFGFGILLLELI

TGLRALEFGKTVSQKGAMLEW

30 VRKLHEEMKVEELLDRELGTNY
DKIEVGEMLQVALLCTQYLPAH
BRKMSEVVIMLE

GDGLAERWAASHNHSHFYHANI
35 SFKTISSLSTTSVSRLDAHCNDPTYQMFG

SSAFDDDDDHQPLDSFAMELSGPR (SEQ ID NO: 59)

Arabidopsis thaliana RKS12 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 tttaaaaaccttqctaqttctcaattctcatgactttqcttttaqtcttagaagtggaaa

ATGGAACATGGATCATCCCGTGGCTTTATTTGGCTGATTCTATTTCTCGATTTTGTTTCC AGACTCACCGGAAAACACAACTTGATGCTCTCATTGCTCTAAGAAGCAGTTTATCATCA GGTGACCATACAAACAATATACTCCAAAGCTGGAATGCCACTCACGTTACTCCATGTTCA TOGTTTCATCTTACTTGCAATACTGAAAACAGTGTTACTCGTCTTGACCTGGGGAGTGCT 15 AATCTATCTGGAGAACTGGTGCCACAGCTTGCTCAGCTTCCAAATTTGCAGTACTTGGAA CTTTTTAACAATAATATTACTGGGGAGATACCTGAGGAGCTTGGCGACTTGATGGAACTA GTAAGCTTGGACCTTTTTGCAAACAACATAAGCGGTCCCATCCCTTCCTCTTTGGCAAA CTAGGAAAACTCCGCTTCTTGCGTCTTTATAACAACAGCTTATCTGGAGAAATTCCAAGG TCTTTGACTGCTCTGCCGCTGGATGTTCTTGATATCTCAAACAATCGGCTCAGTGGAGAT 20 ATTCCTGTTAATGGTTCCTTTTCGCAGTTCACTTCTATGAGTTTTGCCAATAATAATTA AGGCCGCGACCTGCATCTCCTTCACCATCACCTTCAGGAACGTCTGCAGCAATAGTAGTG AAAAGGTTCTCCTTGCGTGAACTGCTAGTTGCTACAGAGAAATTTAGCAAAAGAAATGTA 25 TTGGGCAAAGGACGTTTTGGTATATTGTATAAAGGACGTTTAGCTGATGACACTCTAGTG GCTGTGAAACGGCTAAATGAAGAACGTACCAAGGGTGGGGAACTGCAGTTTCAAACCGAA GTTGAGATGATCAGTATGGCCGTTCATAGGAACTTGCTTCGGCTTCGTGGCTTTTGCATG ACTCCAACTGAAAGATTACTTGTTTATCCCTACATGGCTAATGGAAGTGTTGCTTCTTGT 30 CTGGGATCAGCAAGGGGGCTCGCATATTTACACGATCATTGCGACCAAAAGATCATTCAC CTGGATGTGAAAGCTGCAAATATACTGTTAGATGAAGAGTTTGAAGCTGTTGTTGGAGAT TTTGGGCTAGCAAAATTAATGAATTATAACGACTCCCATGTGACAACTGCTGTACGGGGT ACGATTGGCCATATAGCGCCCGAGTACCTCTCGACAGGAAAATCTTCTGAGAAGACTGAT GTTTTTGGGTACGGGGTCATGCTTCTCGAGCTCATCACTGGACAAAAGGCTTTCGATCTT GCTCGGCTTGCAAATGATGATGATATCATGTTACTCGACTGGGTGAAAGAGGGTTTTGAAA GAGAAGAAGTTGGAAAGCCTTGTGGATGCAGAACTCGAAGGAAAGTACGTGGAAACAGAA GTGGAGCAGCTGATACAAATGGCTCTGCTCTGCACTCAAAGTTCTGCAATGGAACGTCCA AAGATGTCAGAAGTAGTGAGAATGCTGGAAGGAGATGGTTTAGCTGAGAGATGGGAAGAA TGGCAAAAGGAGGAGATGCCAATACATGATTTTAACTATCAAGCCTATCCTCATGCTGGC ACTGACTGGCTCATCCCCTATTCCAATTCCCTTATCGAAAACGATTACCCCTCGGGGCCA

5

Predicted amino acid sequence of the Arabidopsis thaliana RKS12 protein.

Different domains are spaced and shown from the N-terminus

10 towards the C-terminus. Overall domain structure is similar as
described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 2 leucine residues, each

15 separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain

20 contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for Oglycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown

25. function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single

30 leucine rich repeat, probably involved in protein / protein interactions.

MEHGSSRGFI WLILFLDFVSRVTGKTQV

35

DALIALRSSLSSGDHTNNILQ SWNATHVT PCSWFHVTCNTENSVTRL

DIGSANLSGELV

P QLAQLPNLQYLELFNNNITGEI

5 PEELGDLMELVSLDLFANNISGPI

PSSLGKLGKLRFLRLYNNSLSGEI

PRSLTALP LOVLDISNNRLSGDI

PVNGSFSQFTSMRFA NNKLRPR

10 PASPSPSPSGGTS

AAIVVGVAAGAALLFALAWWL

RRKLQGHFLDVPAAEEDPE

15 VYLGQFKRFSLRELLVAT

EKFSKRNVLGKGRFGILYKGRLAD

DTLVAVKRLNEERTKGGELQFQ

TEVEMISMAVHRNLLRLRGFCM

20 TPTERLLVYPYMANGSVASCLR

ERPEGNPALDWPKRKHIALGSA

RGLAYLHDHCDQKIIHLDVKAA NILLDEEFEAVVGDFGLAKLMN

YNDSHVTTAVRGTIGHIAPEYL

25 STGKSSEKTDVFGYGVMLLELI

TGQKAFDLARLANDDDIMLLDW VKEVLKEKKLESLVDAELEGKY

VETEVEQLIQMALLCTQSSAME

RPKMSEVVRMLE

30

GDGLAERWEEWQKEEMPIHDFNYQAY

PHAGTDWLIPYSNSLIENDYPSGPR (SEQ ID NO: 61)

Arabidopsis thaliana RKS13 cDNA

The start codons encoding predicted the methionine residue of the gene product has been indicated by bold capitals. The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

taataaacctctaataataatggctttgcttttactctgatgacaagttcaaaaATGGAA

10 CAAAGATCACTCCTTTGCTTCCTTTATCTGCTCCTACTATTCAATTTCACTCTCAGAGTC GCTGGAAACGCTGAAGGTGATGCTTTGACTCAGCTGAAAAACAGTTTGTCATCAGGTGAC CCTGCAAACAATGTACTCCAAAGCTGGGATGCTACTCTTGTTACTCCATGTACTTGGTTT CATGTTACTTGCAATCCTGAGAATAAAGTTACTCGTGTTGACCTTGGGAATGCAAAACTA TCTGGAAAGTTGGTTCCAGAACTTGGTCAGCTTTTAAACTTGCAGTACTTGGAGCTTTAT 15 AGCAATAACATTACAGGGGAGATACCTGAGGAGCTTGGCGACTTGGTGGAACTAGTAAGC TTGGATCTTTACGCAAACAGCATAAGCGGTCCCATCCCTTCGTCTCTTGGCAAACTAGGA AAACTCCGGTTCTTGCGTCTTAACAACAATAGCTTATCAGGGGAAATTCCAATGACTTTG ACTTCTGTGCAGCTGCAAGTTCTGGATATCTCAAACAATCGGCTCAGTGGAGATATTCCT GTTAATGGTTCTTTTTCGCTCTTCACTCCTATCAGTTTTGCGAATAATAGCTTAACGGAT 20 CTTCCCGAACCTCCGCCTACTTCTACCTCTCCTACGCCACCACCACCTTCAGGGGGGCAA ATGACTGCAGCAATAGCAGGGGGAGTTGCTGCAGGTGCAGCACTTCTATTTGCTGTTCCA GCCATTGCGTTTGCTTGGTGGCTCAGAAGAAAACCACAGGACCACTTTTTTGATGTACCT GCTGAAGAAGACCCAGAGGTTCATTTAGGACAACTCAAAAGGTTTACCTTGCGTGAACTG TTAGTTGCTACTGATAACTTTAGCAATAAAATGTATTGGGTAGAGGTGGTTTTGGTAAA 25 GTGTATAAAGGACGTTTAGCCGATGGCAATCTAGTGGCTGTCAAAAGGCTAAAAGAAGAA CGTACCAAGGGTGGGGAACTGCAGTTTCAAACCGAAGTTGAGATGATCAGTATGGCCGTT CATAGGAACTTGCTTCGGCTTCGTGGCTTTTGCATGACTCCAACTGAAAGATTACTTGTT TATCCCTACATGGCTAATGGAAGTGTTGCTTCTTGTTTAAGAGAGCGTCCTGAAGGCAAT ${\tt CCAGCACTTGATTGGCCAAAAAGAAAGCATATTGCTCTGGGATCAGCAAGGGGGCTTGCG}$ 30 TATTTACATGATCATTGCGACCAAAAAATCATTCACCGGGATGTTAAAGCTGCTAATATA TTGTTAGATGAAGAGTTTGAAGCTGTTGTTGGAGATTTTGGGCTCGCAAAATTAATGAAT TATAATGACTCCCATGTGACAACTGCTGTACGCGGTACAATTGGCCATATAGCGCCCGAG TACCTCTCGACAGGAAAATCTTCTGAGAAGACTGATGTTTTTGGGTACGGGGTCATGCTT CTCGAGCTCATCACTGGACAAAAGGCTTTCGATCTTGCTCGGCTTGCAAATGATGATGAT 35 ATCATGTTACTCGACTGGGTGAAAGAGGTTTTGAAAGAGAAGAAGTTGGAAAGCCTTGTG GATGCAGAACTCGAAGGAAAGTACGTGGAAACAGAAGTGGAGCAGCTGATACAAATGGCT CTGCTCTGCACTCAAAGTTCTGCAATGGAACGTCCAAAGATGTCAGAAGTAGTGAGAATG CTGGAAGGAGATGGTTTAGCTGAGAGATGGGAAGAATGGCAAAAGGAGGAGATGCCAATA 40 AATTCCCTTATCGAAAACGATTACCCCTCGGGTCCAAGATAAccttttagaaagggtctt

ttcttgtgggttcttcaacaagtatatatatagattggtgaagttttaagatgcaaaaaa aa (SEQ.ID.NO: 62)

5

Predicted amino acid sequence of the Arabidopsis thaliana RKS13 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as 10 described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains leucine zipper motifs, containing 2 times 2 leucine residues, each separated by seven other amino acids. The third domain

15 contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to

20 contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine

25 protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein

30 interactions.

MEQRSLLCFLYLL LLFNFTLRVAGNAEG

35 DALTQLKNSLSSGDP ANNVLQSWDATLVT

PCTWFHVTCNPENKVTRV

DLGNAKLSGKLV

P ELGQLLNLQYLELYSNNITGEI

PEELGDLVELVSLDLYANSISGPI

5 PSSLGKLGKLRFLRLNNNSLSGEI

PMTLTSVQLQV LDISNNRLSGDI

PVNGSFSLFTPISFANNSLTDLPE

PPPTSTSPTPPPPSG

10

GQMTAAIAGGVAAGAAL LFAVPAIAFAWWL

RRKPQDHFFDVPGAEEDPE

15 VHLGQLKRFTLRELLVAT

DNFSNKNVLGRGGFGKVYKGRLAD

GNLVAVKRLKEERTKGGELOFO

TEVEMISMAVHRNLLRLRGFCM

20 TPTERLLVYPYMANGSVASCLR

ERPEGNPALDWPKRKHIALGSA RGLAYLHDHCDOKIIHRDVKAA

NILLDEEFEAVVGDFGLAKLMN

YNDSHVTTAVRGTIGHIAPEYL

25 STGKSSEKTDVFGYGVMLLELI

TGQKAFDLARLANDDDIMLLDW VKEVLKEKKLESLVDAELEGKY

VETEVEQLIQMALLCTQSSAME

RPKMSEVVRMLE

30

GDGLAERWEEWQKEEMPIHDFNYOA

YPHAGTDWLIPYSNSLIENDYPSGPR (SEQ ID NO: 63)

35

Arabidopsis thaliana RKS14 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined. Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 ctqcaccttaqagattaatactctcaagaaaaacaagttttgattcggacaaagATGTTG CAAGGAAGAAGAAGAAAAAAAGAGTTATGCTTTGTTCTTCTAACTTTCTTCTTCTTC TTTATCTGTTTTCTTTCTTCTTCTTCTGCAGAACTCACAGACAAAGTTGTTGCCTTAATA GGAATCAAAAGCTCACTGACTGATCCTCATGGAGTTCTAATGAATTGGGATGACACAGCA GTTGATCCATGTAGCTGGAACATGATCACTTGTTCTGATGGTTTTGTCATAAGGCTAGAA 15 GCTCCAAGCCAAAACTTATCAGGAACTCTTTCATCAAGTATTGGAAATTTAACAAATCTT CAAACTGTATACAGGTTATTGCAGAACAATTACATAACAGGAAACATCCCTCATGAGATT GGGAAATTGATGAAACTCAAAACACTTGATCTCTCTACCAATAACTTCACTGGTCAAATC CCATTCACTCTTTCTTACTCCAAAAATCTTCACAGGAGGGTTAATAATAACAGCCTGACA GGAACAATTCCTAGCTCATTGGCAAACATGACCCAACTCACTTTTTTGGATTTGTCGTAT 20 AATAACTTGAGTGGACCAGTTCCAAGATCACTTGCCAAAACATTCAATGTTATGGGCAAT TCTCAGATTTGTCCAACAGGAACTGAGAAAGACTGTAATGGGACTCAGCCTAAGCCAATG TCAATCACCTTGAACAGTTCTCAAAGAACTAAAAACCGGAAAATCGCGGTAGTCTTCGGT GTAAGCTTGACATGTGTTTGCTTGTTGATCATTGGCTTTGGTTTCTTCTTTTGGTGGAGA AGAAGACATAACAAACAAGTATTATTCTTTGACATTAATGAGCAAAACAAGGAAGAAATG TGTCTAGGGAATCTAAGGAGGTTTAATTTCAAAGAACTTCAATCCGCAACTAGTAACTTC AGCAGCAAGAATCTGGTCGGAAAAGGAGGGTTTGGAAATGTGTATAAAGGTTGTCTTCAT GATGGAAGTATCATCGCGGTGAAGAGATTAAAGGATATAAACAATGGTGGTGGAGAGGTT CAGTTTCAGACAGAGCTTGAAATGATAAGCCTTGCCGTCCACCGGAATCTCCTCCGCTTA TACGGTTTCTGTACTACTTCCTCTGAACGGCTTCTCGTTTATCCTTACATGTCCAATGGC 30 AGTGTCGCTTCTCGTCTCAAAGCTAAACCGGTATTGGATTGGGGCACAAGAAAGCGAATA GCATTAGGAGCAGGAAGAGGGTTGCTGTATTTGCATGAGCAATGTGATCCAAAGATCATT CACCGTGATGTCAAAGCTGCGAACATACTTCTTGACGATTACTTTGAAGCTGTTGTCGGA GATTTCGGGTTGGCTAAGCTTTTGGATCATGAGGAGTCGCATGTGACAACCGCCGTGAGA GGAACAGTGGGTCACATTGCACCTGAGTATCTCTCAACAGGACAATCTTCTGAGAAGACA 35 GATGTGTTCGGGTTTCGGGATTCTTCTCGAATTGATTACTGGATTGAGAGCTCTTGAA TTCGGAAAAGCAGCAAACCAAAGAGGAGCGATACTTGATTGGGTAAAGAAACTACAACAA GAGAAGAAGCTAGAACAGATAGTAGACAAGGATTTGAAGAGCAACTACGATAGAATAGAA GTGGAAGAAATGGTTCAAGTGGCTTTGCTTTGTACACAGTATCTTCCCATTCACCGTCCT AAGATGTCTGAAGTTGTGAGAATGCTTGAAGGCGATGGTCTTGTTGAGAAATGGGAAGCT TCTTCTCAGAGAGCAGAAACCAATAGAAGTTACAGTAAACCTAACGAGTTTTCTTCCTCT

GAACGTTATTCGGATCTTACAGATGATTCCTCGGTGCTGGTTCAAGCCATGGAGTTATCA
GGTCCAAGATGAcaagagaaactatatgaatggctttgggtttgtaaaaaa (SEQ ID NO: 64)

5

Predicted amino acid sequence of the Arabidopsis thaliana RKS14 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus, Overall domain structure is similar as

10 described in Schmidt et al. (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain

- 15 contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to
- 20 contain hydroxy-proline residues, and to be a site for 0-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine
- 25 protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein
- 30 interactions.

MLQGRREAKKSYALFSSTFF FFFICFLSSSSAELTDKV

35 VALIGIKSSLTDP HGVLMNWDDTAVD

PCSWNMITCSDGFVIR

LEAPSONLSGTLSS

SIGNLTNLOTVYRLLONNYITGNI

PHEIGKLMKLKTLDLSTNNFTGQI
5 PFTLSYSKNLHRRV NNNSLTGTI

PSSLANMTOLTFLDLSYNNLSGPV

PRSLAKTFNVMGNSOICPT

GTEKDCNGTQPKPMSITLNSSQR

10 TKNRK

IAVVFGVSLTCVCLLIIGFGFLLWW

RRRHNKQVLFFDINEQNKE

15 EMCLGNLRRFNFKELQSAT

SNFSSKNLVGKGGFGNVYKGCLHD

GSIIAVKRLKDINNGGGEVQFQ

TELEMISLAVHRNLLRLYGFCT
20 TSSERLLVYPYMSNGSVA

SRLKAKPVLDWGTRKRIALGAG

RGLLYLHEQCDPKIIHRDVKAA

NILLDDYFEAVVGDFGLAKLLD

HEESHVTTAVRGTVGHIAPEYL

25 STGQSSEKTDVFGFGILLLELI TGLRALEFGKAANORGAILDW

VKKLOOEKKLEOIVDKDLKSNY

DRIEVEEMVQVALLCTQYLPIH

RPKMSEVVRMLE

30

GDGLVEKWEASSQRAET

NRSYSKPNEFSSS

ERYSDLTDDSSVLVQAMELSGPR (SEQ ID NO: 65)

35

Legends

Figure 1

5

The different domains of the predicted RKS gene product have

the following functions:
The first domain of the predicted protein structure at the N-

terminal end consists of a signal sequence, involved in 0 targeting the protein towards the plasma membrane. Protein cleavage removes this sequence from the final mature protein product (Jain et al. 1994, J. Biol. Chemistry 269: 16306-16310). The second domain consists of different numbers of

leucine zipper motifs, and is likely to be involved in protein protein dimerization. The next domain contains a conserved pair of cystein residues, involved in disulphate bridge formation. The next domain consists of 5 (or in the case of RKS3 only 4) leucine rich repeats (LRRs) shown in a gray colour, likely to be involved in ligand binding (Kobe and

Deisenhofer 1994, TIBS 19: 415-420). This domain is again bordered by a domain containing a conserved pair of cystein residues involved in disulphate bridge formation often followed by a serine /. proline rich region. The next domain displays all the characteristics of a single transmembrane

25 domain (http://qenome.cbs.dtu.dk/services/TMHNM/). At the predicted cytoplasmic site of protein a domain is situated with unknown function, followed by a domain with serine /threonine kinase activity (Schmidt et al. 1997, Development 124: 2049-2062). The kinase domain is followed by a domain

With unknown function whereas at the C-terminal end of the protein part of a leucine rich repeat is positioned, probably involved in protein-protein interactions.

Figure 2

35 Alagnment of the predicted protein sequences of the different RKS gene products from Arabidopsis thaliana with alignX, Vector NTI Suite 5.5 resulted in a phylogenetic tree in which the relative homology between the different RKS members is shown.

Figure 3

10

- 5 Intron-Exon bounderies of the genomic regions on the chromosomes of Arabidopsis thaliana encoding the different RKS gene products. Exons are shown as boxes, whereas intron sequences are shown as lines. Sequences encoding LRR domains are displayed in gray colour, transmembrane regions in black.
- Figure 4.

 Cromosomal location of RKS genes in Arabidopsis thaliana, showing colocalisation with GASA genes.
- 15 Figure 5. A signaling complex comprising molecules of RKS proteins, ELS proteins, NDR/NHL proteins and SBP/SPL proteins.

Figure 6.

Second generation (T2) tobacco seedlings germinated on MS

- 20 medium. Transformations were performed with DNA clone 2212-15, representing the overexpression construct GT-RKS4-s. T2 seedlings derived from T1 plant 15.7 shows co-suppression effects while T1 plant 15.6 shows no obvious changes in level of RKS4. T1 plants 15.9 and 15.3 show overexpression effects.
- 25 Plant 15.7 has the lowest remaining level of RKS4 gene product, whereas plant 15.3 has the highest level of RKS4 gene product.

Figure 7

- O Second generation (T2) tobacco plants. In the upper row the offspring from a co-suppressing T1 plant 15.7 is shown. The middle row shows plants derived from a transgenic T1 plant 15.6 with no clear changes in level of RKS4 is shown while the bottom row shows plants derived from a T1 plant 15.3 in which
- 35 the levels of RKS4 are increased by the introduction of the overexpression construct GT-RKS4-s.

Figure 8

Second generation (T2) tobacco plants. Plants derived from a co-suppressing TI plant 15.7 show a reduction in plant size and a delay in the initiation and outgrowth of primordia. The 5 control empty vector transgenic plants show no visible differences in growth compared with the offspring from the transgenic 15.6 plant, in which the endogenous level of RKS4 gene product was not changed. In the overexpressing plants 15.9 and 15.3 organ size was increased, similar as the number 10 of initiated leaf primordia.

Figure 9

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is decreased (right picture) due to the 15 presence of a transgenic RKS4 antisense construct (GT-RKS4-16a). The left picture shows a wildtype plant of the same age as the transgenic antisense plant, grown under similar growth conditions. Plant size, organ size and number of organ primordia is decreased in the transgenic antisense plant 20 compared with the wildtype control.

Figure 10

decreased.

35

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is decreased (bottom left picture) due to the presence of a transgenic RKS4 antisense construct (GT-RKS4-16a). The upper right picture shows a wildtype flower of the same age as the transgenic antisense flower, grown under similar growth conditions. Total flower size is only slightly decreased in the transgenic antisense flower compared with the control flower, whereas organ size of petals is strongly

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is increased (upper left picture) due to the presence of a transgenic RKS4 overexpressing construct (GT-RKS4-6s). Compared with the wildtype control flower, total

flower size of the transgenic flower is clearly increased. Both sepal and petal organ size is clearly increased compared

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with the control.

For comparison an Arabidopsis thaliana WS plant is shown which has been transformed with a construct encoding the GASA3 gene in sense direction, i.e. overexpressing GASA3.

5 Figure 11.

> Formation of meristematic regions in the hypocotyl of Arabidopsis thaliana WS plants under influence of overexpression of RKS4.

10 RKS4 overexpression results in increases in flower and seed organ size that could be due to increase in cell elongation and/or cell division. In order to analyse the cell division patterns in plants with deregulated RKS4 expression the mitotic activity in transgenic plants was analyzed with the a 15 unstable GUS reporter under the control of a cyclin B1;1 promoter (the Plant Journal 1999 (4) 503-508 Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein). Arabidopsis thaliana WS seedlings with the pCDG construct did not show gus activity (cell division) in 20 hypocotyls (top) whereas the same pCDG line crossed with a constitutive RKS4 construct showed mitotic activity as indicated by GUS-positive cells (bottom); indicating that RKS4

25 Figure 12

In Arabidopsis thaliana WS, the seed size is influenced by changing levels of RKS4 gene product. Constitutive overexpression of RKS4 results in increases in seed size (left) compared with control wildtype seeds (right). Antisense 30 constitutive expression of RKS4 cDNA (middle) results in a decrease in seed size compared with the control (right). Magnification is identical in all photos as shown by the bar size.

overexpression activated mitotic activity in hypocotyls.

35

organ size can be influenced by either modulating cell division or cell elongation or a combination of both. In order to identify the total number of cells and the cell size within an organ the apical site of petals of mature Arabidopsis flowers was investigated. Petal organ size is clearly influenced by modulation of RRS4 gene product levels (bottom row for the flowers from which the apical petal epidermal cells were identified). Epidermal cell size is not changed in transgenic plants compared with the control.

10

Figure 14

Arabidopsis thaliana WS plants in which the endogenous level of RKS10 gene product is increased (right picture) due to the presence of a transgenic RKS10 overexpressing construct. The 15 left picture shows the apical epidermus of a full grown cotyl from an empty vector transgenic seedling of the same age as the transgenic overexpressing cotyl, grown under similar growth conditions.

20 Figure 15

Arabidopsis thaliana WS plants in which the endogenous level of RKS10 gene product is decreased (right picture) due to the presence of a RKS10 antisense construct The left picture shows a wildtype plant of the same age as the transgenic antisense plant, grown under similar growth conditions. Plant size, organ size and number of organ primordia remains similar in both the transgenic antisense plants and the wildtype control.

30 Figure 16

In order to determine organ size variations in transgenic RKS10 transgenic plants compared with empty vector control transgenic plants (pGreen4K), flower organ size was determined of the four open flower stages of Arabidopsis inflorescences.

35 The four successive flower stages are photographed under similar magnifications. No obvious changes in organ length

could be observed in size of sepals, petals, stamen and carpel

between empty vector control flowers (pGreen4K), flowers with an antisense RKS10 construct (a) or plants overexpressing the RKS10 cDNA under the control of a 35S promoter (S

5 Figure 17

Tissue cultured auxin treated transgenic Arabidopsis T2
seedlings were grown on MS agar plates without hormones for a
period of 3 weeks. Regeneration potential was scored and the
formation and outgrowth of multiple shoot apical meristems
10 from single seedling origin was displayed as (+). The
formation and outgrowth of only one shoot apical meristem,
leading to the formation of a normal rosette of leaves from
individual plants was displayed as (-). Positive regeneration
controls consisted of seedlings overexpressing either KNAT1,
15 CUC2, IFT or cycD3. All of these showed an increase of
regeneration capacity (+) compared with a negative control GUS
overexpressing plant pGreen5K (-).
Representative examples of RKS and ELS cDNA overexpressing (s)
or antisense (a) cosuppressing constructs in transgenic plants

Figure 18.

20 are shown in the bottom panels.

Tobacco leaf discs were stably transformed with the RKSO overexpressing construct GT-RKSO-23S and from a single 25 transformation event, large numbers of regeneration plantlets were isolated and subcultured. All of the regenerated plants were potted and flowered. The original transformation event could be kept continuously in tissue culture indefinitely.

30 Figure 19

Seedlings from transgenic Arabidopsis thaliana containing either constructs overexpressing (s) or co-suppressing by antisense (a) the RKS gene products were screened for the appearance of fasciation. Several examples in which fasciation 35 could be routinely observed are shown together with a negative control plant (pGreen5K, overexpressing the GUS gene) in which fasciation could never be observed. Figure 20 - 23

Primary root tips of transgenic Arabidopsis plants (top rows) photographed under similar magnification. The bottom rows show 5 the corresponding seedlings (also between each other under the same magnification). Figure 23 shows the specific Arabidopsis transgenes with a strong increase in root outgrowth.

Figure 24

10 Avarage root length of 10-30 transgenic Arabidopsis T2 seedlings from one T1 transgenic plant is shown.

Figure 25

T3 seedlings are shown from a strong co-suppressing RKS10
15 antisense construct line (T1-4; T2-6; T3 generation) and a
strong overexpressing line (T1-4; T2-6; T3 generation). The
overexpressing line is different and stronger from the one
shown in Figure 4.1-4.5. Pictures are taken under similar
magnifications.

20

25

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Figure 26

T2 seed was germinated on horizontal MS agar plates and pictures were taken under similar magnification of representative examples of the lateral root development from transgenic RKS and ELS transgenic roots.

Figure 27

Pictures taken from transgenic RKS8 or RKS10 overexpressing roots taken directly behind the tip zone. Pictures are taken under same magnification.

Figure 28

Arabidopsis thaliana WS plants in which the endogenous level
of RKS or ELS gene product is modified result in the formation
of new meristem formation and / or outgrowth, resulting in a
complex, bushy inflorescence in transgenic Arabidopsis plants
compared with control empty vector control plants (pGreen4K).

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Overexpression of RKS10 and ELS1 (S) and cosuppression with antisense constructs of RKS8 and also RKS10, result in increased numbers of developing generative meristems. The generative shoots are photographed with similar magnification.

Figure 29

Arabidopsis thaliana WS plants in which the endogenous level of RKS gene product is modified result in the formation of new neristem formation and / or outgrowth, resulting in a complex, bushy inflorescence in transgenic Arabidopsis plants commpared with control empty vector control plants (pGreen4K). The top panel shows adult plants under similar magnification. Compared with the control, RKS10 overexpression results in an extreme bushy phenotypic plant. The results of co-suppressing the RKS8 gene product are less dramatic with respect to the bushiness. However, also in these transgenic plants the number of generative meristems is strongly increased compared with the control. The bottom panel shows the generative shoot in detail under similar magnification.

Figure 30

Schematic drawing of the different flower organs in an empty vector control pGreen4K flower (Left) compared with a complex transgenic flower structure seen in transgenic Arabidopsis plants containing an antisense (a) RKS1O construct. The terminal flower meristem produces 2 sepals, 1 petal, 2 stamen, a carpel which is not a closed structure but open with groups of ovules on the inside and outside of this structure, and stigmatic cells protruding from the top part. Two new flowers are protruding from this structure, containing all flower organs in normal numbers.

Figure 31

35 Schematic drawing of the different flower organs in a complex transgenic flower structure seen in transgenic Arabidopsis plants T1-11 containing an antisense (a) RKS10 construct. The terminal flower meristem produces 1 sepal, 2 petals, 2 stamen, a carpel which is not a closed structure but open with groups of ovules on the inside and outside of this structure, and stigmatic cells protruding from the top part. An undetermined 5 flower meristem is protruding from the open carpel structure and forms a number of new flowers, including normal flowers (right) and another abnormal flower (left) which consists of a flower with half of the sepal, petal and stamen organs formed and a new terminal flower meristem protruding from this structure, developing in structures as seen in Figure 7.5. The stamen contain only small numbers of (viable) pollen compared with whidtype stamen (see also chapter 5).

Figure 32

15 Schematic drawing of the different flower organs in an empty vector control pGreendK flower (Left) compared with a complex transgenic flower structure seen in a transgenic Arabidopsis plant T1-11 containing an antisense (a) RKS10 construct (overview shown in Figure 7.4). The terminal flower meristem produces half the normal number of sepals, petals and stamen. The remaining part of the flower structure has converted into a new structure containing a new stem containing a single organ structure resembling a fusion between a petal and a sepal. On this structure several (viable) pollen grains can be observed.

Figure 33

Schematic drawing of the different flower organs in a complex transgenic flower structure seen in a transgenic Arabidopsis

plant 71-12 containing an antisense (a) RKS10 construct. The terminal flower meristem originating from an undetermined generative meristem is here producing an axillary secondary undetermined meristem (left picture), a single organ resembling a stamen (bottom left), a normal flower and a terminal flower. This terminal flower structure contains 2 normal sepals, 2 normal petals, 2 normal stamen (with only a few viable pollen) and two organs resembling a fusion of

sepals /petals/stamen (see also figure 7.7). From this terminal flower structure two new flowers emerge (in a similar fashion as observed in Figure 7.3) containing normal numbers of flower organs (right photos). At the top of this figure a 5 control inflorescense is shown schematically with terminal flower meristems as normally originate from the generative Arabidopsis thaliana generative meristem.

Figure 34

Schematic drawing and detailed pictures of several of the structures as shown in figure 7.6. At the right the organs resembling a fusion between sepals/petals/stamen are shown with viable pollen sticking out from these structures. At the top left the single stamen-like organ directly protruding from the main stem is shown.

Figure 35

Transgenic Arabidopsis plants overexpressing the RKS13 gene product show a modification of the normal flower inflorescence architecture, somewhat resembling the structures observed in RKS10 antisense plants. A terminal flower containing a normal seed developing silique and a small number of sepals, petals and stamen, develops at least 4 additional terminal flower meristems that develop abnormally themselves, resulting in open carpel structures and modifications of organ structures.

Figure 36

Transgenic plants in which the RKS and / or ELS genes are introduced behind a constitutive 358 promoter in an 30 overexpressing (S) or antisense (a) configuration are analyzed for sterility and characterized further for defects in proper pollen development. As a negative control the normal pollen development of a transgene containing the empty expression vector (p64K) was included. First generation transgenic 35 flowers of RKS10 expressing constructs and second generation control vector and ELS2 are shown under similar magnification.

In detail the stigmatic surface and surrounding stamen, are

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shown under similar magnification, showing the presence or absence of pollen on the stamen or the stigmatic surface.

Detailed description

1.Modifying organ size

5

Plant size is determined by both cell elongation and cell division rate. Modifying either one or both processes results in a change in final organ size. Increasing the level of specific members of the family of RKS genes results in an 10 increase in organ size, growth rate and yield. Modulating plant growth, organ size and yield of plant organs is the most important process to be optimized in plant performance. Here we show that modulating the level of members of the family of the RKS signaling complex is sufficient to modulate these 15 processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL 20 protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKJS 10 gene or functional equivalent thereof. Inactivation of endogenous RKS gene product results in a decrease in plant 25 growth, proving that the normal function of these endogenous RKS gene products is the regulation of growth and organ size. Elevation of the levels of the regulating of the RKS signaling complex in plant cells is provided in order to increase: the size of plant organs

30 the growth rate
the yield of harvested crop
the yield of total plant material
the total plant size

35 Decreasing the levels of endogenous RKS gene product is provided in order to decrease: the size of plant organs 5

the growth rate the total plant size

Results obtained (see also figures 6 to 13)

Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35s promoters. Transgenic plants have been produced in Arabidopsis thaliana and in Nicotiana tabacum. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail. The phenotype observed in transgenic plants with antisense constructs of RKS4 (GT-RKS4-a) could be described as dwarf plants in which all plant organs showed a decrease in organs size and growth rate. Overexpression of RKS4 (GT-RKS4-s) resulted in plants with increased size of organs and an increase in growth rate Since cell size alone was not responsible for the modifications in organ size of

regulation of the cellular divisions during plant growth and organ formation. Overexpression of RKS 4 results in an increase of cellular divisions whereas a decrease in endogenous RKS 4 gene product levels within the plant results in a decrease of cellular division rates.

petals it can be concluded that RKS4 is involved in the

25

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2. Cell division

The mitotic cell cycle in eukarvotes determines the total number of cells within the organism and the number of cells 5 within individual organs. The links between cell proliferation, cell differentiation and cell-cycle machinery are of primary importance for eukaryotes, and regulation of these processes allows modifications during every single stage of development. Here we show that modulating the level of members of the family of the RKS signaling complex is sufficient to modulate these processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKJS 10 gene or functional 20 equivalent Herewith the invention provides a method for modulating the number of cells to be formed within an eukaryotic organism as a whole or for modulating the cell number within individual organs is, which of primary importance in modulating plant developmental processes, 25 especially of arable plants. Here we show that members of the RKS signaling complex are able to regulate the number of cellular divisions, thereby regulating the total number of cells within the organism or different organs.

30 Possible Applications

Elevation of the levels of the regulating RKS signaling complex members in plant cells in order to increase: the size of plant organs the growth rate

35 the yield of harvested crop the yield of total plant material the total plant size

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Decreasing the levels of endogenous RKS signaling complex members in order to decrease:

the size of plant organs

5 the growth rate the total plant size

Results obtained

Overexpression and antisense constructs of full length RKS

10 cDNA clones have been made under the control of 35s
promoters. Transgenic plants have been produced in Arabidopsis
thaliana and in Nicotiana tabacum. Subsequent generations of
stably transformed plants were investigated for phenotypes and
analyzed in detail.

15 Overexpression of RKS 4 results in an increase of cellular divisions whereas a decrease in endogenous RKS 4 gene product levels within the plant results in a decrease of cellular division. Another example of RKS genes involved in cellular proliferation is provided by RKS10. Overexpression of RKS10 20 (S) results in a decrease in apical epidermal cells (Figure

14) compared with control plants containing an empty expression cassette (pGreen4K). Co-suppressing the endogenous RKS 10 gene in plants containing an antisense construct (a)

showed clearly larger epidermal cells as the corresponding cells in wildtype control plants (Figure 15). In contrast to the plant phenotypes shown in RKS4 transgenic plants, no differences in plant or organ size could be observed in the RKS10 transgenic plants or organs. This shows that although the organ size remains constant, the number of cells within

30 these organs is variable due to the differences in size of individual cells. These results indicate that normal RKS4 function within the plant can be described as an activator of cellular division.

Normal RKS10 function also involves an activation process on 15 cellular division rate. This effect is also detectable in the root in the region directly behind the tip zone, where in the RKS10 overexpressing transgenes cellular divisions were

detectable in a region where normally cell proliferation has ceased. The plane of divisions of root cells in these transgenes is also clearly different from the normal plane of root cell division, resulting in clumps of cells with all

5 types of division planes possible. In contrast to RKS4, the final organ size in RKS10 transgenic plants is under the control of other organ size restriction processes, in such a way that the final organ volume remains constant (Figure 16). RKS4 and RKS10 are essentially involved in the same cell cycle activation process, but either addition organ size controlling functions of these RKS genes or the hierarchical order in which they regulate the cell cycle is

15

Literature

different.

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3.Regeneration

Modification the levels of different RKS and ELS genes within 5 plants allows the initiation and / or outgrowth of apical meristems, resulting in the formation of large numbers of plantlets from a single source. A number of gene products that is able to increase the regeneration potential of plants is known already. Examples of these are KNAT1, cycD3, CUC2 and 10 IPT. Here we show that modulation of the endogenous levels of RKS genes results in the formation of new shoots and plantlets in different plant species like Nicotiana tabacum and Arabidopsis thaliana. herewith the invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating apical meristem formation, in particular 20 wherein said gene comprises an ELS1, RKS0, RKS3, RKS4, RKS8 or RKS10 gene or functional equivalent thereof. A direct application of a method according to the invention is the stable or transient expression of RKS and ELS genes or gene products in order to initiate vegetative reproduction. 25 Regeneration can be induced after overexpression of for example RKSO and ELS1; or by co-suppression of for example the endogenous RKS3, RKS4, RKS8 or RKS10 genes. Overexpression or co-suppression of these RKS and ELS gene products can be either transient, or stable by integration of the 30 corresponding expression cassettes in the plant genome.

Results obtained

Overexpression and antisense constructs of full length RKS and ELS cDNA clones have been made under the control of 355 promoters. Transgenic plants have been produced in Arabidopsis thaliana and in Nicotiana tabacum. Subsequent generations of

stably transformed plants were investigated for phenotypes and analyzed in detail.

T2 transgenic seedlings of Arabidopsis were germinated in liquid MS medium supplemented with 1 mg/L 2,4-D for 1 week,

5 followed by extensive washing and plating of the seedlings onto MS agar plates without hormones. Control transgenic seedstocks containing either a negative control vector (pGreen5K); or positive control overexpression constructs of gene products known to increase the regeneration potential 10

(IPT, KNAT1, CUC2 and cycD3) were characterized for regeneration potential together with seedstocks from plants either overexpressing (s) or co-suppressing (a) all RKS and ELS gene products (Figure 17). Overexpression of the ELS1 and RKSO cDNA clones resulted in an increase of shoot apical

15 meristem formation and outgrowth, whereas antisense constructs (a) of these cDNA clones did not increase the regeneration potential (only increased regeneration results are shown). Antisense constructs of RKS3, RKS4, RKS8 and RKS10 also resulted in an increased formation and outgrowth of apical 20 meristems (Figure 17).

T1 generation Nicotiana tabacum tissue cultures transformed with ELS and RKS gene products in either overexpression (s) cassettes or antisense co-suppression (a) cassettes allowed the regeneration of indefinite number of offspring plants from a single transformed cell origin (Figure 18). An example is shown for the overexpression of the GT-RKS0-23S construct. The resulting plants obtained from one transformation event in general showed no phenotypes. Only a subset of plants

displayed RKSO overexpression phenotypes (like loss of apical

30 dominance and early flowering).

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Fasciation is normally a result from an increased size of the

4. Fasciation

5 apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the shoot apical meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to stems in which the number of cells is increased. The invention herewith provides a method for 10 modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating fasciation, in particular wherein said gene comprises an RKSO, RKS3, RKS8 or RKS10 gene or functional equivalent thereof. Here we for example show that modulation of the levels of RKS gene products in plants like Arabidopsis thaliana can result in fasciated stems as shown in Figure 19. 20 A direct application as provided herein is the regulated formation of fasciation in plant species in which such a trait is desired like ornamental plants. Regulation of the initiation and extent of fasciation, either by placing the responsible RKS encoding DNA sequences under the control of stage or tissue specific promoters, constitutive promoters or inducible promoters results in plants with localized or constitutive fasciation of stem tissue. Another application is modulating the number of primordiae by regulation of the process of fasciation. An example is provided by for example sprouts, in which an increased number of primordia will result in an increased numbers of sprouts to be harvested. Fasciation can also result in a strong modification in the structural architecture of the inflorescence, resulting in a terminal group of flowers resembling the Umbelliferae type (an example is shown in Figure 19 where the fasciated meristem of a RKSO-7S Arabidopsis plant in which endogenous RKSO gene product

levels have been deregulated clearly terminates in an Umbelliferae type inflorescence.

Results obtained

- 5 Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 355 promoters. Transpenic plants have been produced in Arabidopsis thaliana. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail.
- 10 T2 transgenic seedlings of Arabidopsis were germinated on MS agar plates without hormones. Control transgenic seedstocks containing a negative control vector (pGreen5%) were tested for their ability to induce fasciation (Overexpression constructs (s) of RKSO, RKS8 and RKS10 cDNA clones resulted in 15 fasciated plants, whereas antisense constructs (a) of these
- discitated plants, whereas antisense constructs (a) of these cDNA clones did not increase the regeneration potential (only positive results are shown). Antisense constructs of RKS3 gave also rise to fasciation (Figure 19).

20

Literature

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5. Root development

Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the 5 number of cells within the proliferating zone of the root apical meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to roots in which the number of cells is increased. Adaptation to soil conditions is possible by regulation of 10 root development of plants. Here we describe several processes in root development that can be manipulated by modification of the levels of the RKS signaling complex within the root. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene 15 or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating root development, in particular wherein said gene comprises an ELS1, ELS2, RKS1, 20 RKS3, RKS4, RKS6 RKS8 or RKS10 gene or functional equivalent thereof. Root length, a result by either root cells proliferation or elongation, can for example be increased by overexpression of for example RKS3, RKS4, RKS6 and ELS2, or inactivation of the endogenous RKS10 gene product. Root length 25 can also be decreased by decreasing of endogenous RKS1 levels or by strong overexpression of RKS10. The initiation of lateral roots is also regulated by RKS gene products. Overexpression of for example RKS10 can result in a strong increase in the initiation and outgrowth of lateral roots. Co-30 suppression of RKS1 also resulted in the initiation and outgrowth of large numbers of lateral roots. Root hair formation and elongation is important in determining the total contact surface between plant and soil. A strong increase of root hair length (elongation) can be obtained by 35 overexpression of ELS1 and RKS3 gene products. As the roots of terrestrial plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant

hormones, interaction with the rhizosphere and storage functions, increasing or decreasing root length, for example for flexible adaptations to different water levels, can be manipulated by overexpressing or cosuppressing RKS and / or 5 ELS gene products. Modulation of the total contact surface between plant cells and the outside environment can be manipulated by regulation lateral root formation (increased by RKS10 overexpression and co-suppression of RKS1). Finally the contact surface between plant cells and the soil can be 10 influenced by modulation of the number of root hairs formed or the elongation of the root hairs, as mediated by ELS1 and RKS3.

Results obtained

15 Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in Arabidopsis thaliana. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail. 20 T2 transgenic seedlings of Arabidopsis were germinated on MS agar plates without hormones. Control transgenic seedstocks containing a negative control vector pGreen4K (empty expression vector) and / or pGreen5K (a GUS overproducing vector) were included as references for normal root development. Seedlings from transgenic Arabidopsis thaliana containing either constructs overexpressing (s) or cosuppressing by antisense (a) the RKS gene products were screened for the appearance of fasciation. Several examples in which fasciation could be routinely observed are shown 30 together with a negative control plant (pGreen4K, containing an expressing cassette without an insert cDNA). Seedlings are germinated and grown on vertically placed MS agar plates.

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6. Apical meristems

All parts of the plant above the ground are generally the result on one apical shoot meristem that has been initiated 5 early at embryogenesis and that gives rise to all apical organs. This development of a single meristem into complex tissue and repeated patterns is the result of tissue and stage-dependent differentiation processes within the meristems and its resulting offspring cells. The control of meristem 10 formation, meristem identity and meristem differentiation is therefore an important tool in regulating plant architecture and development. Here we present evidence the function of RKS and ELS gene products in regulation of the meristem identity and the formation and outgrowth of new apical meristems. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and 20 RKS/ELS ligand protein allowing modulating meristem identity, in particular wherein said gene comprises an ELS1, RKS8, RKS10 or RKS13 gene or functional equivalent thereof. Introduction of for example the RKS10 gene product or an other member of the RKS signaling complex under the control of a tissue and / or stage specific promoter as provided herein allows localized 25 and time regulated increases in the levels of gene product. For example the meristematic identity in a determined meristem might thereby be switched back into an undetermined meristem. thereby changing for example a terminal flower into an 30 undetermined generative meristem. Another application might be found in changing the meristematic identity at an early time point, during early vegetative growth, thereby switching the vegetative meristem into a generative meristem, allowing early flowering. 35 Modulation of meristem identity in terminal primordia, like for example as shown in Figure 30, where flower organ primordia are converted into terminal flower primordia, allows

the formation of completely new types of flowers and fused fruit structures. Constitutive overexpression of RKS gene products results in plants with many apical meristems, as Can clearly been seen in Figure 29, where RKS10 overexpression 5 results in an extremely bushy phenotype.

Results obtained

Changing the normal levels of endogenous RKS10 within the 10 plant, either by overexpressing or co-suppressing the RKS10 cDNA, results in an increase in generative meristem development (Figure 28). Compared with the control empty vector transgenic pGreen4K plants, large number of meristems are initiated at places were 15 normally no meristems initiate and / or develop. A clear example is shown by co-suppressing the RKS8 gene (Figure 29), where many new inflorescence meristems are initiated from the central generative meristem compared with control pGreen4K plants of the same age. This phenotype is even more extreme in 20 RKS10 overexpressing plants where the resulting plants are extremely bushy with very large numbers of generative meristems formed. Inactivation of the endogenous RKS10 gene in Arabidopsis results in modification of meristematic identity as can be shown in Figure 30. A determined flower meristem 25 develops into two new normal terminal flower meristems and a number of terminal flower organ primordia. Another example is shown in Figure 31 where meristem determination is switched from a terminal flower meristem, that normally result only in the normal numbers of terminal organ primordia, towards a 30 number of organ primordia, a new undetermined generative meristem that develop into normal flowers or in a new terminal flower meristem with developmental abnormalities. Only half of the terminal flower primordia develop normally while an extra structure arises resembling a new flower stem with a 35 petal/stamen like organ. The few pollen detectable on this structure (Figure 32) were able to pollinate a MS1 (male sterile) Arabidopsis flower. Figure 33 shows the meristematic

developmental switch from a terminal flower meristem into a new undetermined generative meristem, that gives rise to a new formation of another undetermined meristem, and several normal and abnormal terminal flowers. The abnormal flowers again show 5 the fusion of different structures, in this case from sepals, petals and stamen together (Figure 34). Surprisingly, directly on the generative stem another structure, resembling a single stamen was detectable. All these data indicate that a decrease in RKS1 expression levels results in switches in the 10 meristematic identity. Meristems can switch forward and backward between developmental stages, indicating that RKS10 is normally involved in regulating the meristematic identity and the developmental order of meristematic development. RKS13 seems to be involved in similar processes, as can be concluded 15 from the switches in flower meristematic outgrowths observed in figure 35. Modification of the expression levels of RKS1 also results in modified meristem identity. Suppression of endogenous RKS1 levels results in a developmental switching of generative meristems towards vegetative meristems, together 20 with other phenotypes (results not shown).

Literature

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7. Male sterility

Male sterility is a highly desired trait in many plant species. For example, manipulation of pollen development is 5 crucial for F1 hybrid seed production, to reduce labour costs and for the production of low-environmental impact genetically engineered crops. In order to produce hybrid seed from inbred plant lines, the male organs are removed from each flower, and pollen from another parent is applied manually to produce the 10 hybrid seed. This labour-intensive method is used with a number of vegetables (e.g. hybrid tomatoes) and with many ornamental plants. Transgenic approaches, in which one or more introduced gene products interfere with normal pollen initiation and development is therefore highly desired. 15 Especially when the number of revertants (growing normal pollen) is extremely low. Male sterility in plants is a desired trait that has been shown already in many plant species as a result of the inactivation of expression of a number of genes essential for 20 proper stamen development, mitotic divisions in the pollen stem cells, or male gametogenesis. A method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling 25 complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating pollen development, in particular wherein said gene comprises an ELS2 or RKS10 gene or functional equivalent thereof. 30 Here we present data that show that overexpression of gene products, like transmembrane receptor kinases (RKS) and extracellular proteins (ELS) can also result in the formation of male sterility. The ability to induce male sterility by

overexpressing specific genes as provided herein allows the opportunity to produce transgenic overexpressing plants in which the pollen development is inhibited. Stable single copy homogygous integration of such overexpressing traits into the

plant genome will render such plants completely sterile,
making them excellent material for the production of F1 hybrid
seed. Furthermore, the combined integration of a male
sterility inducing overexpressing gene coupled directly with
another desired transgene result in transgenic plants which
are unable to produce transgenic seed, making these transgenic
plants excellent material for outside growth without problems
affecting transgenic pollen spreading throughout the
environment, thereby eliminating possible crosses

combination of a desired transgene flanked on both sites by different male-sterility inducing overexpressing genes would decrease the frequency of pollen formation to an extremely low level. An example is an overexpressing construct of RKS10 at the 5'end of integrated DNA fragment, the desired transgene

expression cassette in the middle and at the 3'end of the integrated DNA the ELS2 overexpressing construct. This complete DNA fragment is integrated into the genome by conventional techniques, like particle bombardment,

20 Aarobacterium transformation etc. Another possible application

concerns the modification of pollen in ornamental plant
species like lily, where the release of pollen from cut
flowers can be avoided by making transgenic plants in which
pollen development is initiated by release from the stamen is
prevented (a desired trait that can be obtained by
overexpressing for example ELS2, resulting in partial pollen
development). Hereby the ornamental value of the stamen with
pollen is not lost, but release of pollen is inhibited.

30 Results obtained

Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 355 promoters. Transgenic plants have been produced in Arabidopsis thaliana. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail.

T2 transgenic seedlings of Arabidopsis were germinated on MS agar plates without hormones. Control transgenic plants

containing a negative control vector pGreen4K (empty expression vector) were included as references for normal stamen and pollen development. RKS10 and ELS2 resulted in sterile plants when overexpressed in Arabidopsis. Antisense 5 RKS10 plants resulted in a strong reduction in the number of pollen formed (Figure 36). In order to determine whether pollen development itself was the reason for sterility (and not a combination of pollen developmental mutants coupled to either embryo lethals or female gametogenesis defects), 10 reciprocal crosses were performed between sterile transgenic plants and wildtype Arabidopsis thaliana WS plants. These results confirmed that the sterile plants with overexpressing RKS10 and ELS2 constructs were male sterile but completely female fertile. No defects could be observed in embryo 15 development from crosses between female transgenic overexpressors and male wildtype pollen (results not shown). Since both antisense and overexpressing constructs of the RKS10 gene showed defects in proper pollen development we conclude that normal levels of endogenous RKS10 gene product 20 are essential for proper pollen formation, outgrowth and differentiation. In the ELS2 overexpressing plants the initiation of pollen grains was not inhibited. However the proper development of pollen grains in full grown viable pollen was clearly inhibited .

25

Literature

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Resistance mechanisms

Two-hybrid interaction experiments have already shown in vitro interaction between RKS and NDRO-NHT, and members of the 5 SBP/SPL family. Here we show that in vivo the individual components of this signalling cascade are regulating identical processes, as based on functional genomics on transgenics plants, overexpressing or co-suppressing single components or combinations of components in this transmembrane signalling 10 complex.

Here we show a large number of new members of the NDR/NHL gene family and we postulate a function as syntaxins in the pathogen resistance:

15 At2q27080;

MAERVYPADS PPOSGOFSGN FSSGEFPKKP APPPSTYVIO VPKDOIYRIP PPENAHRFEO LSRKKTNRSN CRCCFCSFLA AVFILIVLAG ISFAVLYLIY RPEAPKYSIE GFSVSGINLN STSPISPSFN VTVRSRNGNG KIGVYYEKES SVDVYYNDVD ISNGVMPVFY QPAKNVTVVK LVLSGSKIQL TSGMRKEMRN EVSKKTVPFK LKIKAPVKIK FGSVKTWTMI VNVDCDVTVD 20 KLTAPSRIVS RKCSHDVDLW ** (SEQ ID NO: 66)

At5g21130

MTVEKPOEMT GDTNSDGFLT NKDVHRIKHP SLDTNDSSSS RYSVDSQKSR IGPPPGTYVI KLPKDOIYRV PPPENAHRYE YLSRRKTNKS

25 CCRRCLCYSL SALLITIVLA ATAFGFFYLV

YOPHKPOFSV SGVSVTGINL TSSSPFSPVI RIKLRSONVK GKLGLIYEKG NEADVFFNGT KLGNGEFTAF KQPAGNVTVI VTVLKGSSVK LKSSSRKELT ESQKKGKVPF GLRIKAPVKF KVGSVTTWTM TITVDCKITV DKLTASATVK TENCETGLSL L* (SEQ ID NO: 67)

30 At1g65690

MSOHOKIYPV ODPBAATARP TAPLVPRGSS RSBHGDPSKV PLNORPORFV PLAPPKKRRS CCCRCFCYTF CFLLLLVVAV GASIGILYLV FKPKLPDYSI DRLOLTRFAL NODSSLTTAF NUTTTAKNIPN EKIGIYYEDG SKITUWYMEH OLSNGSLPKF YOGHENTTVI YVEMTGOTON ASGLETTLEE QOORTGNIPL RIRVNQPVRV KFGKLKLFEV RFLVRCGVFV DSLATNNVIK

35 IOSSSCKFRL RL* (SEQ ID NO: 68)

At5q36970

MSDHOKIHPV SDPEAPPHPT APLVPRGSSR SEHGDPTKTO QAAPLDPPRE KKGSRS CWCRCVCYTLLVLF LLIVIVGAIV GILYLVFRPK FPDYNIDRLQ LTRFQLNQDL

40 SLSTAFNVTI

TAKNPNEKIG IYYEDGSKIS VLYMOTRISN GSLPKFYOGH ENTTIILVEM TGFTONATSL MTTLQEQQRL TGSIPLRIRV TQPVRIKLGK LKLMKVRFLV RCGVSVDSLA ANSVIRVRSS NCKYRFRL* (SEQ ID NO: 69)

45 At1g54540

50

MGDOOKIHPV LOMEANKTKT TTPAPGKTVL LFVORPIPPP VIPSKNRNMC CKIFCWVLSL LVIALIALAI AVAVVYFVFH PKLPSYEVNS LRVTNLGINL DLSLSAEFKV EITARNPNEK IGIYYEKGGH IGVWYDKTKL CEGPIPRFYO GERNVTKLNV ALTGRAOYGN TVLAALOOOO OTGRVPLDLK VNAPVAIKLG NLKMKKIRIL GSCKLVVDSL STNNNINIKA SDCSFKAKL* (SEQ ID NO: 70)

MADLNGAYYG PSIPPPKKVS HSHGRRGGGC GCLGDCLGCC GCCILSVIFN ILITIAVLLG IAALIIWLIF RPNAIKFHVT DAKLTEFTLD PTNNLRYNLD LNFTIRNPNR RIGVYYDEIE VRGYYGDORF GMSNNISKFY OGHKNTTVVG TKLVGOOLVL LDGGERKDLN EDVNSQIYRI

DAKLELKIRF KFGLIKSWEF KPKIKCDLKV PLTSNSTSGF VFQPTKCDVD F** (SEQ ID NO: 71)

At5q11890

5

MTDRVFPASK PPTATNGAPP VGSIPPPPAP ATVTSNGTIN GMANOKPOVY IPANRPVYRP QPYSRRHHHQ SRPSCRRICC CCCFWSILII LILALMTATA ATAMYVIYHP RPPSFSVPSI RISRVNLTTS SDSSVSHLSS FFNFTLISEN PNOHLSFSYD PFTVTVNSAK SGTMLGNGTV 10 PAFFSDIGNK TSFHGVIATS TAARELDPDE AKHLRSDLTR ARVGYEIEMR TKVKMIMGKL

KSEGVRIKUT CEGFEGTIPK GKTPIVATSK KTKCKSDLSV KVWKWSF* (SEQ ID NO: 72)

Atla17620

MTDDRVYPAS KPPAIVGGGA PTTNPTFPAN KAQLYNANRP AYRPPAGRRR TSHTRG 15 CCCRCCCWTIFVII LLLLIVAAAS AVVYLIYRPO RPSFTVSELK ISTLNFTSAV

IARNPNKNVG FIYDVTDITL YKASTGGDDD VVIGKGTIAA FSHGKKNTTT LRSTIGSPPD BLDBISAGKL KGDLKAKKAV AIKIVLNSKV KVKMGALKTP KSGIRVTCBG IKVVAPTGKK

ATTATTSAAK CKVDPRFKIW KITF** (SEQ ID NO: 73) 20

At3g11650

MGSKOPYLNG AYYGPSIPPP PKAHRSYNSP GFGCCCFSCL GSCLRCCGCC ILSLICNILI AVAVILGVAA LILWLIFRPN AVKFYVADAN LNRFSFDPNN NLHYSLDLNF TIRNPNORVG 25 VYYDBFSVSG YYGDORFGSA NVSSFYOGHK NTTVILTKIB GONLVVLGDG ARTDLKDDEK SGIYRINAKL RLSVRFKFWF IKSWKLKPKI KCDDLKIPLG SSNSTGGFKF QPVQCDFDLS** (SEQ ID NO: 74)

At2q22180

MEGPRRPPSA TAPDSDDDKP DDPPSVWHRP TSSLPALPSL DPPSHGSHHW RNHSLNLSPL 30 PTTSSPPLPP PDSIPELETY VVOVPRDOVY WTPPPEHAKY VEKRSKNPEK NKKKGCSKRL LWFFIILVIF GFLLGAIILI LHFAFNPTLP VPAVERLTVN PSNFEVTLRA ENPTSNMGVR YMMEKNGVVS LTYKNKSLGS GKFPGLSQAA SGSDKVNVKL NGSTKNAVVQ PRGSKQPVVL MLNMELKAEY EAGPVKRNKE VVVTCDVKVK GLLDAKKVEI VSENCESEFK N* (SEQ ID NO: 75)

35 At5g22870

MCHKPKLELM PMETSPAOPL RRPSLICYIF LVILTLIFMA AVGFLITWLE TKPKKLRYTV ENASVONENL TNDNHMSATE OFTIOSHNEN HRISVYYSSV EIFVKFKDOT LAFDTVEPFH OPRMNVKOID ETLIAENVAV SKSNGKDLRS ONSLGKIGFE VFVKARVRFK VGIWKSSHRT AKIKCSHVTV SLSQPNKSQN SSCDADI* (SEQ ID NO: 76)

40

At2q35980 MAAEOPLNGA FYGPSVPPPA PKGYYRRGHG RGCGCCLLSL FVKVIISLIV ILGVAALIFW LIVRPRAIKF HVTDASLTRF DHTSPDNILR YNLALTVPVR NPNKRIGLYY DRIBAHAYYE GKRESTITLT PFYOGHKNTT VLTPTFOGON LVIFNAGOSR TLNAERISGV YNIBIKFRLR 45 VRFKLGDLKF RRIKPKVDCD DLRLPLSTSN GTTTTSTVFP IKCDFDF** (SEQ ID NO: 77)

At2g46300

MADYOMNPVL OKPPGYRDPN MSSPPPPPPP IQQOPMRKAV PMPTSYRPKK KRRSCCRFCC CCICITLVLF IFLLLVGTAV FYLWFDPKLP TFSLASFRLD GFKLADDPDG ASLSATAVAR 50 VEMKNPNSKL VFYYGNTAVD LSVGSGNDET GMGETTMNGF ROGPKNSTSV KVETTVKNOL VERGLAKELA AKFOSKOLVI NVVAKTKVGL GVGGIKIGML AVNLRCGGVS LNKLDTDSPK CILNTLKWYK IISN* (SEQID NO: 78)

At4q05220

55 MTPDRTTIPI RTSPVPRAQP MKRHHSASYY AHRVRESLST RISKFICAMF LLVLFFVGVI AFILWLSLRP HRPRFHIQDF

VVQGLDQPTG VENARIAFNV TILNPNQHMG VYFDSMEGSI YYKDQRVGLI PLLNPFFQQP TNTTIVTGTL TGASLTVNSN RWTBFSNDRA QGTVGFRLDI

PLLNPFFQQP TNTTIVTGTL TGASLTVNSN RWTEFSNDRA QGTVGFRLDI VSTIRFKLHR WISKHRMHA NCNIVVGRDG LILPKFNHKR CPVYFT* (SEQ ID NO: 79)

5 At2g35460

MANGLINGASY GPPIKPPVKT YYSHGRRGSD VGCGICGCFS SCLLCCGGCL VNIICNILIG VLVCLGVVAL LIWFILRPNV VKFQVTEADL TRFEFDPRSH NLHYNISLNF SIRNFNGRLG HYDOLEVBG YYGDGFSAA NHTSFYGGHK NTTVOTTELN GGKLVLILGAG GERDFERDRR

SGYYRIDVKL RFKLRFKFGF LINSWAVRPKI KCHLKVFLST SSSDERFQFH PTKCHVDL* (SEQ ID NO: 80)

At2g27260

MODDERDATG YPYPYPYPNP QOQOPPINGY PREAACTAYP YQNNINYYAP QEMPEAVILE RLFIVPTTHI LLLGILIPIP FLIVRPQLPD WNINGLSVEN FRVENNOWGE KENDLOLFEN PHENNSLRYE TALCAMYINR VSLSETRIOP FOQKKOQTV VHATLEVSGT YVJGGLUDSI 15 GKREVKGNV EPULRMISYV TERYGAFERR RYVIVYCDDV AVGVPVSSGE GKMVGSSKRC KTY** (SEC IN INC. SI

At4g01410

MGEGEAKAEH AAKADHKNAP SASSTPESYS KEGGGGGGDA RRAICGAIFT ILVILGIIAL
20 ILMIVYEPEK PELTVUGAAI YDLINFTAPPL ISTSVĮFSVI ARIPURRVSI HYDKLSHYVT
YKDQIITPPL PLPPLRIGHK STVVIAPVMG GRGIPVSPSV AMGINDRAS GVVULRVVIF
GELRKKAGAI KTGRYGFYAR CDVURRVRPS SNGQVPLLAP STCKVDV* (SEG ID NO: 82)

At5g22200

25 MTGRYCDQHN GYEERRMUM MRRIAWACIG LIVAVAFVVP LVWAILHPHG PRFVLQDYTI MDRYNGOPUF LESKILQYTVS SRINNDKIGI FYDRIDIYVT YRMGENTLAR LLPSTYGGHL EVTYWSPFLI GSAVPVAPYL SSALMEDLFA GLVLLNIKIG GWYRWKYGSW VSGSYRLHVN CPAFITYTGK LIGGGPAIKY QLVQRGAYDV * (SEQ ID NO:83)

30 At1g61760

MENKVDSLPV RSNPSTRPIS RHHSASNIVH RVKBSLTTRV SKLICAIFLS LLLCLGIITF ILMISLOPHR PRVHIRGPSI SGLSRPDOPE TSHISPKITA HENPONVOITY YDDWGSGYYY EKERIGSTKL TNPFYQDPKN TSSIDGALSR PAMAVNKDRW MEMERDRNQG KIMPRLKYRS MIRYKYYTWH SKSHKWYASC YIBIGWGGML LSATKOKKCP YVFT* (SEC) ID NO: 84)

35 At3g52470

MSKOCKHIGG GKEVVVRLC AAIIAFIVIV LITIFLWWI LRPTKPRFVL QDATVYAPNL SQPNLLTSNF QVTLASRNEN SKIGIYDRL HVYATYMRQQ ITLETAIPPT YQQHEGWNW SPFVYQTAVP IAPYNSVALG EEKORGFVGL MIRADGTVRW KVRTLITGKY HIHVRCQAFI 40 NLSWRAAGVL VGDNAVKTIL ANKCSWN** (SEQ [D NO: 85]

At5g53730

MSQISITSPK HCAKKGGINI NNRHKKLFFT FSTPFSGLLL IIFLVWLILH PERPEFSLTE
ADIYSLMITT SSTELLINSSV QLILFSRNFH KXVGIYYDKL LVVAAYRGQQ ITSEASLPPF
45 YQSHEBINLI TAFLQGTELP VAQSPGYQIS RERSTGKIII GMKMDGKLRW KIGTWVSGAY
RFNNYCLAIV AFGMNNTTPP LASLGGTECS TIT* (SEQ ID NO: 86)

At4q01110

MAGETLIKEV LOKEPGYERE HSOPOTPIGS SSSSSSHURE PEKHALPAAP YPTKERONSE O CRUFCCCVCI TVALVILLLI LIVEVEPHLY SPELDVURLS SERVENNYBE GGKADGLIGO LITARATARLD FENDROKKLEY YYGNUDVAVS VOEDDFETSL GSTKYKGFVE KPGNETUVIV PIKYKKQQUD DPIVKELBAD MKSKKLIVVKY MAKTKYGIGV GERKIVIVGV TISCGGVRLQ TLDSRWSKCT IRMLKNYPLG UKKZY. (SEG [D NC: 87]

55 At2g35960

MTTKDCGNHG GGGGGGTASR ICGVIIGFII IVLITIFLVW IILQPTKPRF ILQDATVYAF

MLSQPNLLTS NFQITIASRN RNSRIGIYYD RLHVYATYRN QQITLRTAIP PTYQGHKEDN VWSPFVYGNS VPIAPFNAVA LGDEQNRGFV TLIIRADGRV RWKVGTLITG KYHLHVRCQA FINLADKAAG VHVGENAVKY MLINKCSVNV * (SEQ ID NO: 88)

5 At3q52460

MPSPPREETQ PKPDTGPGQN SERDINGPPP PPPQSQPPPP QTQQQTYPPV MGYPGYHQPP PPYPNYPNAP YQQYPYAQAP PASYYGSSYP AQQNPVYQRP ASSGFVRGIF TGLIVLVVLL CISTTITWLV LRPQIPLFSV NNFSVSNFNV TGPVFSAQWT ANLTIENQNT KLKGYFDRIQ GLVYHQNAVG EDEFLATAFF QPVFVETKKS VVIGRTLTAG DKEQPKVPSW VVDEMKKERE

10 TGTVTFSLRM AVWVTFKTDG WAARESGLKV FCGKLKVGFE GISGNGAVLL PKPLPCVVYV* (SEQ ID NO: 89)

At4g09590

MITKECGNHG GGGGGGGTAC RICGAIIGFI IIVLMTIFLV WIILOPKNPR FILODTTVYA FNLSQPNLLT SKFQITIASR NRNSNIGIYY DHLHAYASYR NQQITLASDL PPTYQRHKED 15 SVWSPLLYGN QVPIAPFNAV ALGDEQNSGV FTLTICVDGQ VRWKVGTLTI GNYHLHVRCQ AFINOADKAA GVHVGENTVK YTLINKCSVN F* (SEQID NO: 90)

At2g35970

MTTKECGNHG GGGGGGGTAC RICGALIGFI IIVLMTIFLV SIILOPKKPE FILODTTVYA 20 FNLSQPNLLT SKFQITIASR NRNSNIGIYY DHLHAYASYR NQQITLASDL PPTYQRHKEN SVWSPLLYGN QVPIAPFNAV ALGDEQNSGV FTLTICVDGR VRWKVGTLTI GNYHLHVRCQ AFINQADKAA GVHVGENTVK YTLINKCSVN F* (SEQ ID NO: 91)

At3g26350

25 MSHHHHHETN PHFARIPSON PHLKSGGAST SQTSSNOPHI PPIPHPKKSH HKTTOPHPVA PPGILIKTRG RHRENPIQEP KHSVIPVPLS PEERLPPRKT QNSSKRPLLL SPEDNQQQRP PPPQAPQRNG GGYGSTLPPI PKPSPWRTAP TPSPHHRRGP RLPPPSRETW AMTWSAAFCC AIFWVILILG GLIILIVYLV YRPRSPYVDI SAANLNAAYL DMGFLLNGDL TILANVTNPS KKSSVEFSYV TFELYYYNTL IATOYIEPFK VPKKTSMFAN VHLVSSOVOL OATOSRELOR 30 QIETGPVLLN LRGMFHARSH IGPLFRYSYK LHTHCSVSLN GPPLGAMRAR RCNTKR* (SEQ ID NO: 92)

At3g11660

MKDCENHGHS RRKLIRRIFW SIIFVLFIIF LTILLIWAIL QPSKPRFILQ DATVYAFNVS GNPPNLLTSN FOITLSSRNP NNKIGIYYDR LDVYATYRSQ QITFPTSIPP TYQGHKDVDI 35 WSPFVYGTSV PIAPFNGVSL DTDKDNGVVL LIIRADGRVR WKVGTFITGK YHLHVKCPAY INFGNKANGV IVGDNAVKYT FTTSCSVSV** (SEQID NO: 93)

At3g44220

MTEKECEHHH DEDEKMRKRI GALVLGFLAA VLFVVFLVWA ILHPHGPRFV 40 LQDATIYAFN VSQPNYLTSN LQVTLSSRNP NDKIGIFYDR LDIYASYRNO QVTLATLLPA TYQGHLDVTI WSPFLYGTTV PVAPYFSPAL SQDLTAGMVL LNIKIDGWVR WKVGTWVSGR YRLHVNCPAY ITLAGHFSGD GPAVKYQLVQ RCAVDV* (SEQ |D NO: 94)

At1g08160

45 MVPPNPAHQP ARRTQPQLQP QSQPRAQPLP GRRMNPVLCI IVALVLLGLL VGLAILITYL TLRPKRLIYT VEAASVQEFA IGNNDDHINA KPSYVIKSYN PEKHYSVRYH SMRISTAHHN QSVAHKNISP FKQRPKNETR IETOLVSHNV ALSKFNARDL RABKSKGTIE MEVYITARVS YKTWIFRSRR RTLKAVCTPV MINVTSSSLD GFQRVLCKTR L** (SEQ ID NO: 95)

50 At2g01080

MPPPPSSRA GLNGDPIAAQ NQQPYYRSYS SSSSASLKGC CCCLFLLFAF LALLVLAVVL IVILAVKPKK POFDLOOVAV VYMGISNPSA VLDPTTASLS LTIRMLFTAV NPNKVGIRYG ESSFTVMYKG MPLGRATVPG FYQDAHSTKN VRATISVDRV NLMQAHAADL VRDASLNDRV BLTVRGDVGA KIRVMNFDSP GVQVLLPSFL PAFCSLSDLA * (SEQ ID NO: 96)

55

At5g06330

MTSKDCGSHD SHSSCNRKIV IWTISIILLL ILVVILLVWA ILQPSKPRFV LQDATVFNFN VSGNPPNLLT SNFOFTLSSR NPNDKIGIYY DRLDVYASYR SQQITLPSPM LTTYOGHKEV NVWSPFVGGY SVPVAPYNAF YLDQDHSSGA IMLMLHLDGR VRWKVGSFIT GKYHLHVRCH

ALINFGSSAA GVIVGKYMLT ETCSVSV* (SEQ ID NO: 97)

5

At5q56050 MSKFSPPPQS QPQPPETPPW ETPSSKWYSP IYTPWRTTPR STQSTPTTTP IALTEVIVSK SPLSNQKSPA TPKLDSMRAH PLHETMVLLQ LRTSRTNPWI WCGAALCFIF SILLIVFGIA

TLILYLAVKP RTPVFDISNA KLNTILFESP VYFNGDMLLO LNFTNPNKKL NVRFENLMVE 10 LWFADTKIAT QGVLPFSQRN GKTRLEPIRL ISNLVFLPVN HILBLRRQVT SNRIAYEIRS

NFRVKAIFGM IHYSYMLHGI CQLQLSSPPA GGLVYRNCTT KRW* (SEQ ID NO: 98)

At3q20600

NDR1

15 MNNQNEDTEG GRNCCTCCLS FIFTAGLTSL FLWLSLRADK PKCSIQNFFI PALGKDPNSR DNTTLNFMVR CDNPNKDKGI YYDDVHLNFS TINTTKINSS ALVLVGNYTV PKFYOGHKKK AKKWGQVKPL NNQTVLRAVL PNGSAVFRLD LKTQVRFKIV FWKTKRYGVE VGADVEVNGD GVKAQKKGIK MKKSDSSFPL RSSFPISVLM NLLVFFAIR* (SEQ ID NO: 99)

20 At3q54200

MSDFSIKPDD KKEEEKPATA MLPPPKPNAS SMETQSANTG TAKKLRRKRN CKICICFTIL LILLIAIVIV ILAFTLFKPK RPTTTIDSVT VDRLQASVNP LLLKVLLNLT LNVDLSLKNP NRIGFSYDSS SALLNYRGQV IGEAPLPANR IAARKTVPLN ITLTLMADRL LSETQLLSDV MAGVIPLNTF VKVTGKVTVL KIFKIKVQSS SSCDLSISVS DRNVTSQHCK YSTKL* (SEQID NO: 100)

25

non-race specific disease resistance protein, putative MTKIDPEEEL GRKCCTCFFK FIFTTRLGAL ILWLSLRAKK PKCSIONFYI PALSKNLSSR DNTTLNPMVR CDNPNKDKGI YYDDVHLTFS TINTTTTNSS DLVLVANYTV PKFYOGHKKK AKKWGQVWPL NNQTVLRAVL PNGSAVFRLD LKTHVRFKIV FWKTKWYRRI KVGADVEVNG DGVKAQKKGS KTKKSDSSLP LRSSFPIFVL MNLLVFFAIR * (SEQ ID NO: 101)

At4g39740

MSHVTATSLA RFTKPVPKPA SSPIVNTKLT TSGGRTAAFM DLSSFRLTVW DPDTANDSSG KFPWPRFLFF FLTLKTGGSG LNIKPTISAI AOMMNPMTIT EMNNOMHRLE QKLLLFLPGS LFLRLSTILH YPGEGSNRPD PLEHALRRSR SLGLDQEEAA KKVIRVGRDS KNDYVNVVEN QAASFLRRCG PSKRIOSVNY CKSTROGHEI PDVKPLFPTG GGTQAPSRSR ARYAVPAILL GFAGFVGFLH YNDERRAVPR GQASSNSGCG CGSNTTVKGP IIGGPFTLVS TENKIVTEND 40 FCGKWVLLYF GYSFSPDVGP EQLKMMSKAV DKLAILLNPL TFGCLYLYAE FDSRILGLTG TASAMROMAQ EYRVYFKKVQ EDGEDYLVDT SHNMYLINPK MEIVRCFGVE YNPDELSQEL LKEVASVSQ* (SEQ ID NO: 102)

At1g32270 syntaxin, putative

45 MVRSNDVKFQ VYDAELTHFD LESNNNLQYS LSLNLSIRNS KSSIGIHYDR FEATVYYMNQ RLGAVPMPLF YLGSKNTMLL RALFEGQTLV LLKGNERKKF EDDOKTGVYR IDVKLSINFR VMVLHLVTWP MKPVVRCHLK IPLALGSSNS TGGHKKMLLI GOLVKDTSAN LREASETDHR RDVAQSKKIA DAKLAKDFEA ALKEFOKAQH ITVERETSYI PFDPKGSFSS SEVDIGYDRS QEQRVLMESR 50 RQEIVLLDNE ISLNEARIEA REQGIQEVKH OISEVMEMFK DLAVMVDHOG TIDDIDEKID NLRSAAAQGK SHLVKASNTQ GSNSSLLFSC SLLLFFFLSG DLCRCVCVGS ENPRLNPTRR KAWCEEEDEE QRKKOOKKKT MSEKRRREEK KVNKPNGFVF CVLGHK* (SEQ ID NO: 103)

MSHHYETNP HFVQFSLQDQ HQGGPSSWM SPHHUQIPQA HSVAPPRVKI KTGGRÄDTE PETIHESESS REJEREEP LEPRENBAS REJQLSEEQ RPHRGYGSE PTPWRAPTR PAYQGGFRT KPHTLPATIC CALILLIVLII SGILLLIVVI AMPREPSFYFI ISAATLAYTAN LOMGYVINGD LAVVNIFNP SKKSSVDESY VMFELYFYNT LIATEHIEFF IVEKGMSHT SFHLWSGVVI LOMIGSQDLQ LQLGTGFVLL NIRGTFHARS NLGSLMRYSY WLHTQCSISL NTPPRACTHAR RRCYNTR'S (SEC IO NO: 104)

At5g45320

MPRITSRHGT SPFIWCAAII CAIISIVVIV GGIIVFVGYL VIHPRVPIIS

VADAHLDFLK YDIVGVLQTQ LITVIRVEND NAKAHALFDE TEFKLSYECK
PIAILKAPEF EVVKEKSMFL PYLVQSYPIP LNPTMQAVD YAVKKUVITF
ELKGGSRTEW RVGPLGSVKF ECNLSCQLRF RPSDHSYIPS PCTSAHKM* (SEQ ID NO: 105)

At3g20610

MORDDAMEMF VTIVGSLMTL LYVSFLLALC LWLSTLVHHI PRCSIHYFYI PALNKSLISS DNTTLNFMVR LKNINAKQGI YYEDLHLSFS TRINNSSLLV ANYTVPRFYQ GHEKKAKKWG ORTFLNYGYV IQAVLPNGSA IFRVDLKMQV KYKVMSWKTK RYKLKASVH EVNEDGATKV KOKEDGIKMK ISDSSPGRUT FFQVCFSIIC VLMNWLIFLA IR* (SEQ ID NO: 106)

20 At4g26490

MYLTKPATVR FNGLDAEPRK DRVILROPRS SRTSLWIWCV AVFLAIRPRI PVFDIPNANL HITYPDTPEE FNOLSKLVN FYNPIKKIEV KFEKLRIELF FENKLIAAGV VOFFLOKKHE TRLEPIRLIS SLVGLPVNHA VELRROLENN KIEYEIRGTF KVKAHFGMIH YSYQLHGRCQ LONTOPPEG LISRNCTTKK * (SEO ID NO: 107)

At5g42860

MHARTDSEVT SISASSPTRS PRRPAYFVQS PSROSHDGEK TATSFHSTEV
LTSPMGSPPH SHSSSSFSK INGSKREGAG GEKCPAHEE EGLIDDDDRE
QBALPRRCYV LAFIVGFSLL FAFFSLILYA AAKPQKFKIS VKSITFGUK
QGAGDAGGI GTDMITMANT LRMLYRNTGT FFGVHYTSSF IDLSFSGITI
GGGIKKFYO SKKSGRTVVV NVLGBKLPLY GGGSTLVPPP PFAPIPPFKK
KKGFIVIVEP PAPPAPPMR LHFTVRSRAY VLGKLVQPKF YKRIVCLINF
EHKKLSKHLF ITNNCTYTEI * (SEG ID NC: 108)

35 At1g45688

MHAKTDSEVT SLAASSPARS PRRPVYYVQS PSRDSHDGEK TATSFHSTPV LSPMGSPPHS
HSSMGRHSRR SSSSRFSGSL KRGSKRVNEN DGSKRKGHGG EKOMKECAVI EEGGLLDDG
ROGGVPRECV VLAFIVGFFI LEGFESLLIV GAAKPHKPKH ITKSITFETH KJQAGQDAGG
VGTDMITMNA TLRMLYRNTG TFFGVHVTST PIDLSFSQIK IGSGSVKKFY QGRKSERTVL
VHVICEKIPL YGSGSTLDEP PEPALEPREV KKKGAPVPIP DEPAPPARVE MILSEVVRSR
AVYLGKLVDG KYKKECDI NEREKNINKH UYTKNOVTT VF' (SFO ID NO: 109)

At4a26820

MODEONIVEE NNOCLLITVI DTEKVPELBP ISSKSHOESE PANISHNSLI FKLFLAITIM

GACVAGYTV LILTEPTEVT WYGSMISFA NNINDFWSAT FSIKMPENE, HVYENFSWU

LVHRGKLVST ARADSFWOKG GEKMETIVER NETKVIDEEA AWEMEDEVAV TGGVVGLDAV

FSGRVGFYFG TSALMGEQYM SAVCENVSAK LYNVDDEIYG TNRSVLSFDG RLVCSVRLEK

YF* (SEQ.ID NO:110)

50

Plants respond in a variety of ways to pathogens. After a recognition of the pathogen, normally mediated by avr and R genes, the resulting response induces a hypersensitive

response, that results in inhibition of the pathogen. After the recognition, further processes appear to be non-specific. In addition to the hypersensitive response, a second line of defence, defined as the systemic acquired resistance response 5 can be triggered, that renders unaffected parts of the plant resistant to a variety of normally virulent pathogens. Several of the RKS and ELS gene products prove to be key regulators in the regulation of the system acquired resistance response.

- 10 Overexpression of several of the RKS and / or ELS genes in plants, either by constitutive promoters, stage and / or tissue specific promoters, or inducible promoters allows the activation of a systemic acquired resistance response in plants.
- 15 Another application can be provided by the activation of a RKS /ELS specific ligand in (transgenic) plants, thereby activating the receptor complex, that finally results in triggered activation of the systemic acquired resistance response in these plants.
- overexpression of an essential regulatory gene in systemic acquired resistance. H. Cao et al. 1998. Proc. Natl. Acad. Sci. USA 95: 6531-6536). Recent literature shows the functional interaction between RKS10 and BRI-1, another class of transmembrane LRR receptor kinases (Cell Vol. 110, 213-222

20 (ref. Generation of broad-spectrum disease resistance by

- 2002). BAK1-RKS10 as descibed here, interacts with BRI-1 and modulates brassinosteroid signaling; Cell vol 110, 203-212 2002 BRI1/BAK1 a receptor kinase pair mediating brassinosteroid signaling). Brassinosteroids are known to
- 30 function in a broad range of disease resistance in tobacco and rice (Plant Journal 2003, 887-898). The BRT-1 receptor is involved in the binding of systemin, an 18 amino acid polypeptide, representing the primary signal for the systemic activation of defence genes (PNAS 2002, 9585-9590).
- 35 ELS overexpression phenotypes mimic the effects of inactivation of RKS molecules gene products. Either ELS is competing for ligand binding, or ELS inhibits the interactions

between RKS and BRI-1-like gene products. ELS1 overexpression results in dwarf phenotypes in Arabidopsis and tobacco plants, similar as observed for antisense RKS4 and RKS10, and for knock out plants of RKS0 and RKS4.

5 Deregulating expression of ELS and / or RKS genes in plant would modify the broad spectrum disease resistance in such plants. This would explain the observed data that brassinosteroids are involved in disease resistance (Plant Journal 2003, 33 887-898.)

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Claims

 A method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein or encoding a protein comprising a ligand for said complex.

10

- 2. A method according to claim 1 allowing modulating cellular division during plant growth or organ formation
- A method according to claim 2 wherein said gene comprises
 an RKS4 or RKS10 gene or functional equivalent thereof.
 - 4. A method according to claim 1 allowing modulating apical meristem formation.
- A method according to claim 4 wherein said gene comprises an ELS1, RKS0, RKS3, RKS4, RKS8 or RKS10 gene or functional equivalent thereof.
- A method according to claim 4 allowing modulating 25 fasciation.
 - A method according to claim 6 wherein said gene comprises an RKSO, RKSS, RKSS or RKSSO gene or functional equivalent thereof.

- A method according to claim 4 allowing modulating root development.
- A method according to claim 7 wherein said gene comprises
 an ELS1, ELS 2, RKS1, RKS3, RKS4, RKS6, RKS8 or RKS10 gene or functional equivalent thereof.

- 10. A method according to claim 4 allowing modulating meristem identity.
- 11. A method according to claim 9 wherein said gene comprises 5 an ELS1, RKS8, RKS10 or RKS13 gene or functional equivalent thereof.
 - 12. A method according to claim 1 allowing modulating pollen development.

10

- 13. A method according to claim 11 wherein said gene comprises an ELS2 or RKS10 gene or functional equivalent thereof.
- 15 14. A method for providing resistance to a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising NDR/NHL protein, or encoding a protein comprising a ligand for said complex.

- 15. A method for obtaining a plant or plant cell with a modulated development comprising subjecting a plant or plant cell to a method according to anyone of claims 1 to 13.
- 25 16. A method for obtaing a resistant plant or plant cell comprising subjecting a plant or plant cell to a method according to claim 14.
- A plant or plant cell obtainable with a method according
 to claim 15 or 16.

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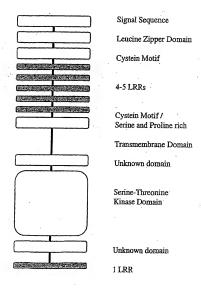
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Fig. 1

Different domains of RKS proteins

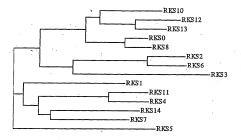


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Fig. 2

Developmental tree of the different Receptor Kinases like SERK (RKS) genes.

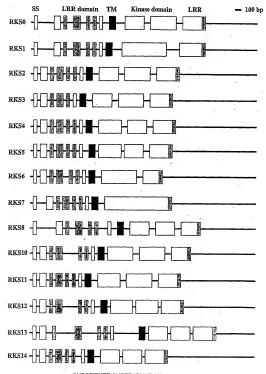


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Fig. 3

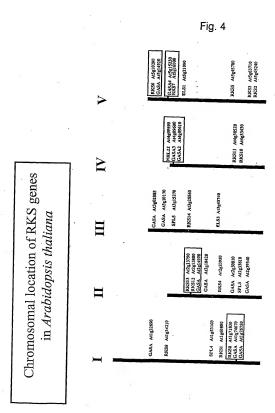
Intron-Exon structure of the RKS genes in Arabidopsis thaliana var. Columbia. SS signal sequence; LRR leucine rich repeat domain; TM transmembrane domain.



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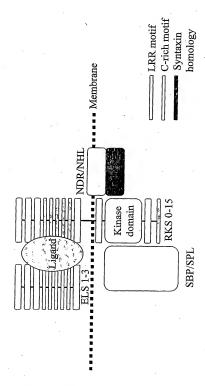


RKS-mediated signal transduction pathway in plants

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Fig. 5



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Modifications in the

expression proficle of GT-RKS4 modulates organ size within seedlings of *Nicotiana tabacum*.



in Nicotiana tabacum.

GT-RKS4 determines seeling size

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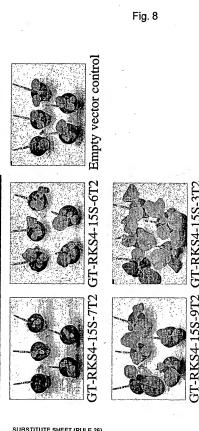
Fig. 7

GT-RKS4-7S-T2

GT-RKS4 determines organ size in Nicotiana tabacum.

GT-RKS4 determines plant size in Nicotiana tabacum 8/36

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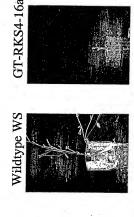


Stable transformed GT-RKS4-antisense

in Arabidopsis thaliana

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Fig. 9



Overexpression of antisense GT-RKS4-1a reduces plant and organ size.

16: 111

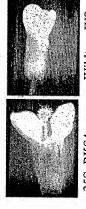
WO 2004/007712

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Fig. 10

Ectopic expression of RKS4 and GASA3 flower size in Arabidopsis thaliana WS gene products both result in increases



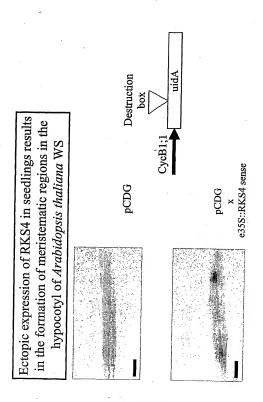




e35S::GASA3 sense

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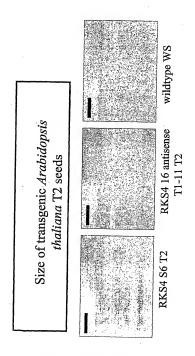
Fig. 11



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Fig. 12



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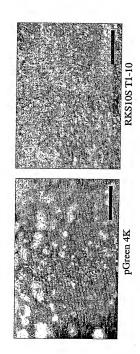
Fig. 13

flower petal Apical site antisense RKS4 regulates cell number and cell size in Arabidopsis thaliana. overexpression Empty vector control

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Fig. 14



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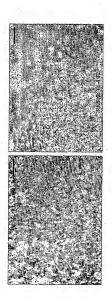
RKS10S T1-10 results in a decrease in size of cotyl-like apical epidermal cells

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Fig. 15

RKS10antisense T1-4 results in an increase in size of the cotyl epidermal cells



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Fig. 16

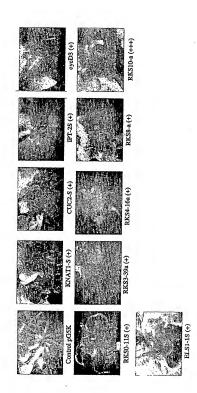
Flower development from the same influorescense in transgenic Arabidopsis thaliana

Regeneration potential of Arabidopsis transgenic seedlings.

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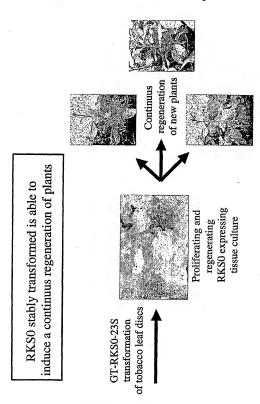
Fig. 17



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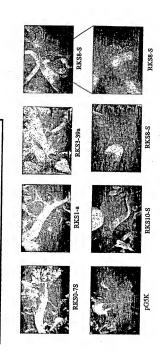
Fig. 18



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Fig. 19

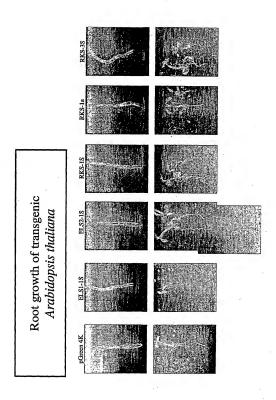


Arabidopsis thaliana

Fasciation in transgenic

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Fig. 20



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Fig. 21

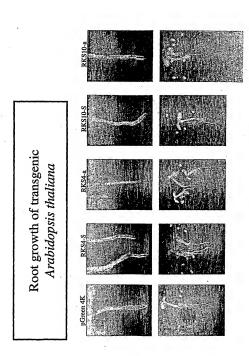
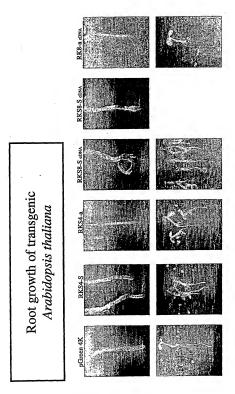


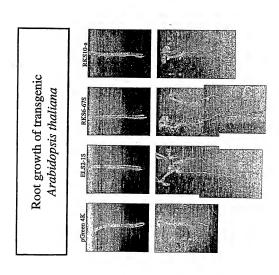
Fig. 22



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Fig. 23

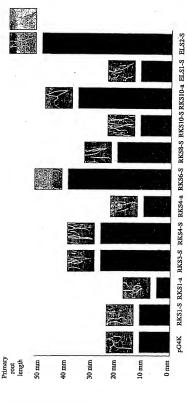


Transgenic construct

Transgenic Arabidopsis thaliana primary root length after 14 days of germination

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Fig. 24

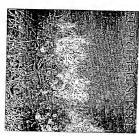


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Fig. 25

Effects of RKS10 transgenic constructs on plant development of 45 days old *Arabidopsis* WS

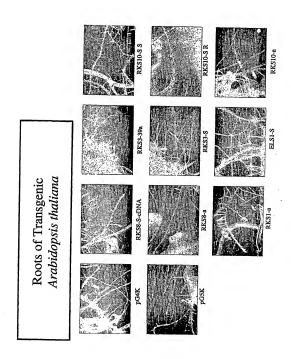




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Fig. 26



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Fig. 27







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Fig. 28





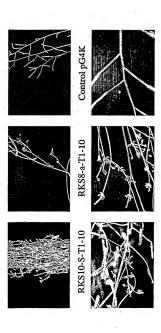


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Fig. 29

Influorescences of T1 transgenic Arabidopsis WS plants

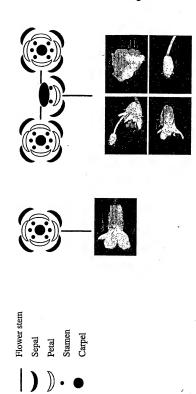


RKS10a T1 expression constructs in Arabidopsis thalinana

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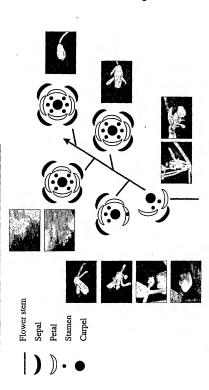
Fig. 30



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Fig. 31



RKS10a T1-11 in Arabidopsis thalinana

RKS10 antisense effects in Arabidopsis thaliana

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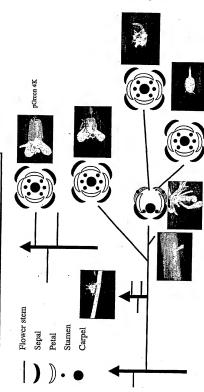
Fig. 32

RKS10a T1-11 pGreen 4K

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Fig. 33

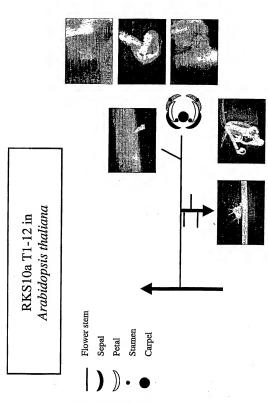


RKS10a T1-12 in Arabidopsis thalinana

6.4.45

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Fig. 34



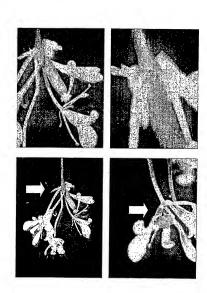
47.65

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Fig. 35

RKS13 regulates flower meristem identity in Arabidopsis thaliana



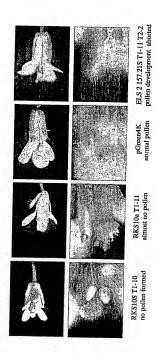
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Fig. 36

Male sterile transgenes in Arabidopsis thaliana



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